

GLUCOKINASE ACTIVATORS: A GLUCOSE SENSOR ROLE IN PANCREATIC ISLETS AND HEPATOCYTE

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ABSTRACT

Glucokinase is a hexokinase isoenzyme and it is a monomer. The glucose –sensing enzyme Glucokinase (GK) is expressed in multiple organs and plays a key role in hepatic glucose metabolism and pancreatic insulin secretion. GKs stimulate insulin release and glucose metabolism in the liver there by lowering blood sugar and are highly effective in patients with type –II diabetes mellitus. GK was an outstanding drug target for developing anti diabetic medicines because it has an exceptionally high impact on glucose homeostasis, because of its glucose sensor role in pancreatic β -cells and as a rate controlling enzyme for hepatic glucose clearance. Glucose homeostasis is maintained by the balanced secretion and action of insulin on one side and glucagon, epinephrine, cortisol, and growth hormone on the other. The secretion of these hormones is governed in turn by glucose sensor cells, most often with glucokinase as the glucose sensor. glucose sensing can be either direct as in the case of the insulin –producing β -cells or indirect as in the case of the epinephrine - releasing chromaffine cells, which are stimulated by nicotine cholinergic neurons that are controlled by glucose sensor of the central and autonomic nervous system.

Keywords: Type-II diabetes, GK (Glucokinase), Homeostasis

INTRODUCTION

Enzymes are biocatalysts synthesized by living cells Involved in several chemical react that are involved in chemical reactions. A kinase is also known as a phosphotransferase. This is a type of enzyme that transfers phosphate groups from high-energy donor molecules, such as ATP to specific substrates. The process is referred to as phosphorylation. Kinases bind substrate proteins and ATP and transfer a phosphate group from ATP to amino acids with free hydroxyl group. The products are phosphor protein and ADP. Kinases act on serine or threonine and tyrosine. The phosphate group is negatively charged and changes the substrate protein in different ways. These changes can be reversed by a separate class of enzymes called phosphatases that remove the phosphate groups^[1,2].

Glucokinase (GK) is an enzyme fourth isoform of hexokinase (EC 2.7.1.1)

ISOFORMS OF HEXOKINASE

They are designated hexokinases I, II, III, and IV or hexokinases A, B, C, and D.

- Hexokinase I/A: It is found in all mammalian tissues, and is considered a “housekeeping enzyme,”unaffected by most physiological, hormonal, and metabolic changes.
- Hexokinase II/B: It constitutes the principal regulated isoform in many cell types and is increased in many cancers.
- Hexokinase III/C: It is substrate-inhibited by glucose at physiologic concentrations. Little is known about the regulatory characteristics of this isoform.

Hexokinase IV/D (“GLUCOKINASE”): It is a mammalian hexokinase IV, also referred to as glucokinase, differs from other hexokinases in kinetics and functions. Human hexokinase IV, hexokinase D, and ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1. The location of the phosphorylation on a subcellular level occurs when glucokinase translocates between the cytoplasm and nucleus of liver cells. Glucokinase can only phosphorylate glucose if the concentration of this substrate is high enough; its Km for glucose is 100 times higher than that of hexokinases I, II, and III. It is monomeric, about 50kD, displays positive cooperativity with glucose, and is not allosterically inhibited by its product, glucose-6-phosphate. ^[3]

Diabetes mellitus

Diabetes mellitus is referred to as it is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced.

There are three main types of diabetes:

- **Type 1 diabetes:** Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack ^[4]
- **Type 2 diabetes:** Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor.
- **Gestational diabetes:** It occurs in combination of relatively inadequate insulin secretion and responsiveness and in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women develop type 2 diabetes later in life.^[5]

Symptoms:

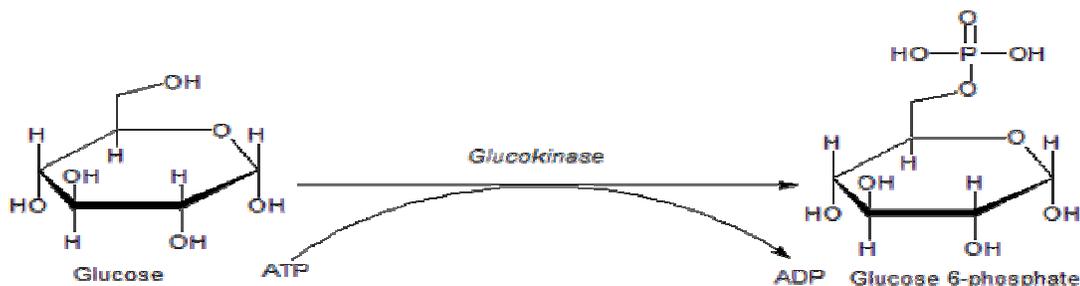
Polyuria (frequent urination),
Polydipsia (increased thirst) and
Polyphagia (increased hunger).

Causes:

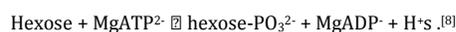
Symptoms may develop rapidly (weeks or months) in type 1 diabetes while in type 2 diabetes they usually develop much more slowly and may be subtle or absent.

- Endocrinopathies
 - Cushing syndrome
 - Hyperthyroidism
 - Pheochromocytoma
- Infections
 - Cytomegalovirus infection
 - Coxsackievirus B
- Genetic defects in insulin processing or insulin action
 - Defects in Thyroid hormone^[6]

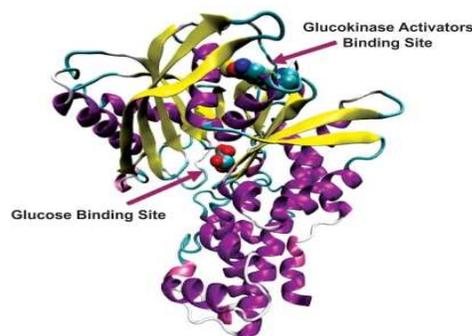
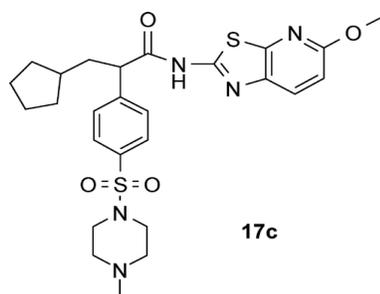
Several combination products have shown to be effective in treatment of type 2 diabetes. Several factors are of importance in choosing the best agent, such as tolerability, effectiveness, contraindicating disease, and side effects profile. Also patient cofactors play an additional important role. The main purpose of this paper is to review different oral hypoglycemic agents, their use, and effectiveness and compare them to the new approved therapies: sitagliptin (Januvia), exenatide (Byetta), and the new inhalation technology in diabetes management Exubera.^[7]



General Glucokinase reaction is more accurately described as:



CHEMICAL STRUCTURE RIBBON STRUCTURE



FUNCTION OF GLUCOKINASE

- Glucokinase is the glucose sensing enzyme in the liver and the pancreas. Glucokinase is responsible for phosphorylating the majority of glucose in the liver and pancreas.
- Glucokinase only binds to and phosphorylates glucose when levels are higher than normal blood glucose level, allowing it to maintain constant glucose levels. By phosphorylating glucose, glucokinase creates glucose 6-phosphate and it can be used by the liver through the glycolytic pathway. Through this the majority of the body's glucose is stored.
- Glucose 6-phosphate is also one of the starting materials of the TCA cycle which is responsible for the majority of ATP production in the bod.^[9]

LOCATION OF GLUCOKINASE ENZYME IN BODY:

Glucokinase is present in the liver, pancreas, hypothalamus, small intestine, and perhaps certain other neuroendocrine cells, and plays an important regulatory role in carbohydrate metabolism.

- In the beta cells of the pancreatic islets, it serves as a glucose sensor to control insulin release, and similarly controls glucagon release in the alpha cells.
- In hepatocytes of the liver, glucokinase responds to changes of ambient glucose levels by increasing or reducing glycogen synthesis.

SUBSTRATES AND PRODUCTS OF GLUCOKINASE:

- Substrate of glucokinase is glucose,
- Product of glucokinase glucose-6-phosphate (G6P).

The other substrate, from which the phosphate is derived, is adenosine triphosphate (ATP), which is converted to adenosine diphosphate (ADP) when the phosphate is removed.

The reaction catalyzed by glucokinase is:

The network of glucokinase (GK)-containing tissues throughout the body and their connections^[10].

Status of Glucokinase (GK) in Diadetes mellitus -II (T2DM):

Hepatic GK expression is insulin-controlled and may be greatly reduced in severe forms of T2DM as demonstrated in human and animal models of the disease with severe insulin deficiency. Still, whatever GK remains available or is newly induced by GKA-stimulated insulin release is probably responsive to activation by GKAs^[11].

BIOLOGICAL ROLE OF GLUCOKINASE ACTIVATORS IN DIFFERENT ORGANS FROM BODY

Glucose and GKA actions on network of sentinel glucokinase cells and parenchymal hepatocytes in glucose homeostasis.^[10] 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. And figure:1. it can see in 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^[12].

Example: they stimulate β -cells but inhibit α -cells, directly or indirectly.

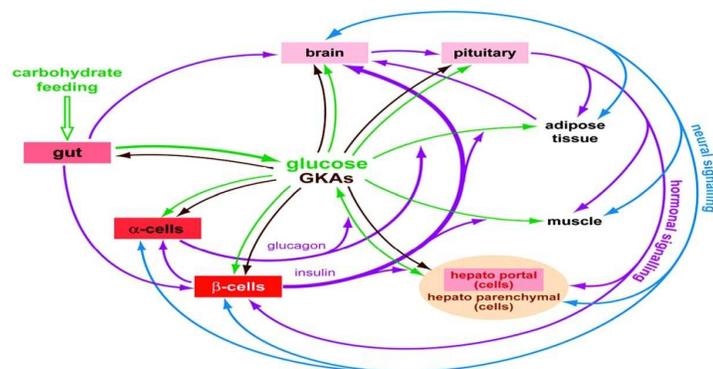


Fig. 1:

ROLE OF GK IN GLUCOSE HOMEOSTASIS

The 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. Endocrine and hepatic glucokinase are encoded by a single gene with two distinct cell-specific promoters:

- An upstream promoter for the cell and
- A downstream promoter for the hepatocyte.
- One of these operates constitutively in GK-containing glucose-sensing endocrine cells (homeostatically most important in the pancreatic β -cell but also demonstrated in pancreatic α -cells, enter endocrine cells, and pituitary gonadotropes) and in neurons of the hypothalamus and the brainstem^[13].
- The other promoter controls hepatic GK expression but in a process absolutely dependent on the presence of insulin.

On recombinant GK inhibited by GK regulatory protein (GKRP), identified a lead molecule that stimulated GK directly but also blocked GKRP inhibition of the enzyme.

GKA ACTION AT THE CELLULAR, MOLECULAR, ORGAN AND WHOLE BODY CELL:

GKAs are, small molecules with a considerable variety of chemical structures that mostly adhere to a common pharmacophore model with related structural motifs. The model consists of a center of a carbon or an aromatic ring with three attachments to it. Two of these are hydrophobic, and at least one of the two is aromatic. The third attachment is a 2-aminoheterocycle or N-acyl urea moiety that provides the basis of forming an electron donor/acceptor interaction with R63 of GK^[14].

Amino acids to be involved in GKA binding: V62, R63, S64, T65, G68, S69, G72, V91, W99, M210, I211, Y214, Y215, M235, V452, V455, and K459^[15].

Most GKAs do not bind to GK in the absence of glucose. GKAs potentiate the competitive glucose reversal of GK inhibition by Glucokinase regulating protein (GKRP). Binding of GKRP and GKAs to GK is mutually exclusive. GKA effects on the known interaction of GK with cellular organelles (e.g., mitochondria or insulin granules) or other macromolecules besides GKRP (e.g., the pro-apoptotic BAD [Bcl-2-associated death promoter] protein or the bifunctional enzyme that generates and hydrolyzes fructose-2, 6-bisphosphate. Impaired formation of a GK/BAD complex proposed to associate with mitochondria and to enhance glucose oxidation may be significant for the molecular pathogenesis of the functional beta-cell defect and apoptotic beta-cell loss known to cause T2DM. Therefore, GKAs are anti-apoptotic in GK-containing cells.

ROLE OF GLUCOKINASE ACTIVATORS IN PANCREATIC β -CELLS:

GK plays this essential role by operating in tandem with the adenine nucleotide-sensitive K channel/SUR1 complex (the K_{ATP}

channel/sulfonylurea-receptor 1 complex) and voltage-sensitive L-type Ca channels that are the other critical components in GK and controls glycolytic and oxidative ATP production and thereby determines the ratio of ATP to ADP, which in turn regulates the K channel, closing it and depolarizing the cell gradually as the ratio increases. The L-type Ca^{2+} channel opens when its membrane potential threshold is reached, which then triggers insulin release by activating several signaling pathways involving Ca^{2+} , cAMP, inositol-3-phosphate, and protein kinase C^[16-20].

Functions of glucokinase in the β -cell:

- Glucokinase is required for GSIR in the physiological range of 4–8 mmol/l.
- 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^[21-24]

GLUCOKINASE-LINKED HYPO- AND HYPERGLYCEMIA SYNDROMES:

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 an activation or inactivation^[25-28] of the enzyme and cause hypo- and hyperglycemia syndromes, respectively. These mutations are collectively described as glucokinase disease. A mild form of hyperglycemia is the result when only one allele is affected by an inactivating mutation, 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^[29-31].

CLASSIFICATION OF GLUCOKINASE ACTIVATORS:

Class-I : Amide based chiral ligands

Class-II : Amide based achiral ligands

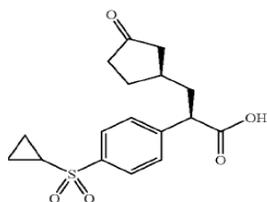
Class-III: Urea derivatives

Class-IV: Miscellaneous ligands

Class I: Amide-Based Chiral GKAs

The three cyclic moieties joined in Y shape contain amide linkage along the stem of the Y, the amide NH acts as hydrogen bond donor and involved in specific hydrogen bond formation with Arg63 backbone carbonyl O. Heterocyclic group containing N at position two connected to the amide NH of ligand makes another specific hydrogen bond formation with the Arg63 backbone NH. This class of

ligands contains cyclopentyl or cyclohexyl (R_3) as one arm of the ligand and shows packing in hydrophobic pocket S. Ligands containing hydrogen bond acceptor group in R_2 makes additional hydrogen bond formation with Tyr215 and Gln98. A Ligand 3 shows three hydrogen bond formations with Arg63 due to presence of ester group in thiazole ring. Val62, Pro66, Ile159, Met210, Ile211, Tyr214, Met235, Cys220, Val452, Val455, Ala456 are the main residues involved in hydrophobic interactions with this class of ligands.^[32-33]

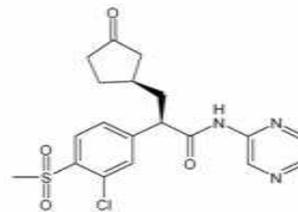


2(R)-(4-Cyclopropanesulfonyl phenyl)-3-(3-oxo-cyclopentyl) propionic acid

Class II: Amide-Based Achiral GKAs

This class of GKAs lacks chiral carbon connected to amide group. Multi-substituted cyclic ring is present which acts as stem of Y letter and substituents on this ring orient in the shape of Y. Lack of chiral carbon center in these ligands makes ligands less flexible. Hydrophobic pocket 2 accommodates smaller hydrophobic groups while hydrophobic pocket 3 accommodates bigger hydrophobic groups. This class also shows specific hydrogen bond formation with the Arg63 backbone. Ligands having carboxyl group attached to

heterocyclic ring shows additional hydrogen bond formation with the Arg63 side chain and contributes to the binding energy. Tyr215 and Gln98 are involved in hydrogen bond formation with ether group. Amino group is present instead of ether group in ligand 10 and this amino group also shows hydrogen bond formation with Tyr215 and Gln98 amino acids.^[34-37]



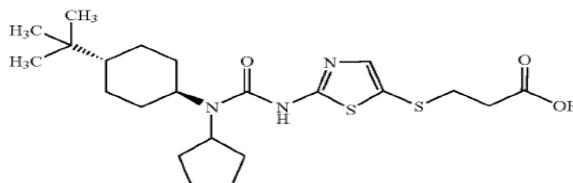
R04389620-000compoundM-Piragliatin

Class III-Urea Derivatives GKAs:

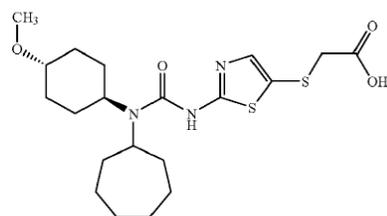
This class of GKAs resembles structurally with class I GKAs. Binding modes of the ligands are also similar in orientation as Class I GKAs. This class of ligands also shows specific hydrogen bond formations with Arg63 carbonyl O and NH to NH of amide and N of thiazole, respectively. R_3 group lies in hydrophobic pocket 3 and makes hydrophobic interactions with Val62, Ile159, Met210, Ile211, Met235, Val452, amino acid residues. Tyr215 is involved in aromatic/hydrophobic interaction at hydrophobic pocket 2 and also forms hydrogen bonds with ligands containing hydrogen bond acceptor group in R_2 .^[38-40]

Dtugs used in urea glucokinase activators:

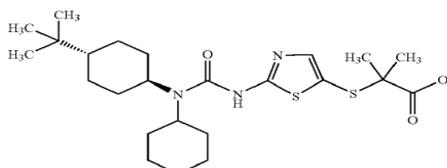
3-{2-[3-(trans-4-tert-Butyl-cyclohexyl)-3-cyclopentyl-ureido]-thiazol-5-ylsulfanyl}-propionic acid



{2-[3-Cycloheptyl-3-(trans-4-methoxy-cyclohexyl)-ureido]-thiazol-5-ylsulfanyl}-acetic acid



2-{2-[3-(trans-4-tert-Butyl-cyclohexyl)-3-cyclohexyl-ureido]-thiazol-5-ylsulfanyl}-2-methyl-propionic acid

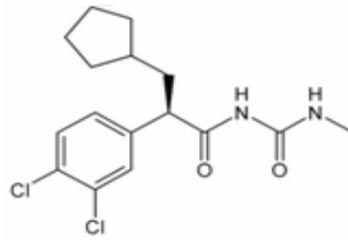


Class IV: Miscellaneous GKAs

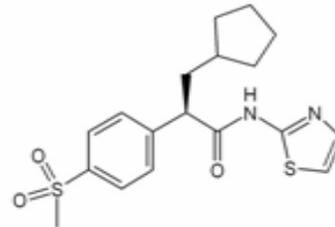
This class of GKAs contains ligands which have some unique groups. Ligand 19 and 20 contains cyclopropyl group connecting three arms. Thr65 is involved in hydrogen bond formation with O of methyl sulfone group. Ligand 18 does not contain amide group, this is structurally different from all other ligands. The ligand also shows

involvement of Arg63 in two specific hydrogen bond formation with N of pyridine and carbonyl group of carboxylic group of ligand 18.

This suggests that Arg63 is highly conserved residue which is specifically involved in hydrogen bond formation with GKAs. Hydrophobic interactions for this class were similar to that of other classes of GKAs.^[40-42]



Compound-A: R00274375-000



Compound-B: R00281675-000

MECHANISM OF GLUCOKINASE ACTIVATORS

Glucokinase-dependent mechanisms in fuel stimulation of insulin release and their modification by neuro-endocrine factors or pharmacological agents. In figure: 2

A: Minimalistic scheme of fuel sensing by the 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.^[43]

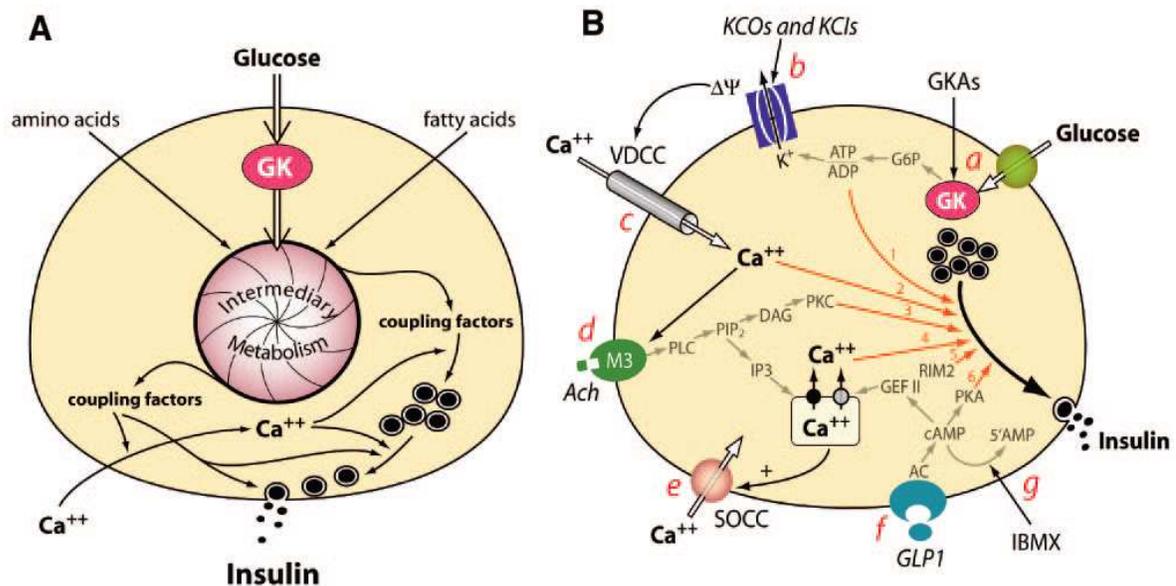


Fig. 2

The role of GK at the molecular, cellular, organ, and whole-body level has to be understood. It is a monomer with a molecular mass of 50 kDa. Its glucose $S_{0.5}$ (the half-saturation concentration) is ≈ 7.0 mmol/L, and the cosubstrate MgATP is saturating physiologically (half-saturated at 0.4 mmol/L). The enzyme shows sigmoidal glucose dependency and thus is most sensitive to glucose changes at ≈ 4.0 mmol/L. The crystal structure of GK has been solved, both as a ligand-free apoenzyme in a "super-open" conformation and as a ternary complex with one d-glucose and a GKA bound in a "closed"^[43] conformation. GK plays this essential role by operating in tandem with the adenine nucleotide-sensitive K channel/SUR1 complex (the K_{ATP} channel/sulfonylurea-receptor 1 complex) and voltage-sensitive L-type Ca^{2+} channel. GK controls glycolytic and oxidative ATP production and thereby determines the ratio of ATP to ADP, which in turn regulates the K channel, closing it and depolarizing the cell gradually as the ratio increases. The L-type Ca^{2+} channel opens when its membrane potential threshold is reached, which then triggers insulin release by activating several signaling pathways involving Ca^{2+} , cAMP, inositol-3-phosphate, and protein kinase C. An alteration of any one of the three critical components of this functional unit influences the threshold for GSIR (glucose stimulated insulin release) profoundly. High glucose induces GK expression in the β -cell concentration dependently as much as five-

to tenfold and thus sensitizes them to glucose stimulation of insulin biosynthesis and release.^[44] The role of insulin in this induction process is debatable. α -Cells are stimulated by amino acids, epinephrine, and acetylcholine. They are strongly inhibited by glucose through complex mechanisms still hotly debated. Glucose inhibition of glucagon secretion could be direct through the GK sensor mechanism or indirect through paracrine effects possibly involving insulin, zinc, or γ -aminobutyric acid. In the liver, GK plays a role that is functionally different from that outlined for the β -cell. High glucose and fructose-1-phosphate (a metabolite of dietary fructose) dissociate the GK/GKRP complex, resulting in translocation of active GK to the cytosol. Hepatic GK regulates glycolysis, the pentose-phosphate shunt, glucose oxidation, and associated oxidative phosphorylation and ATP production. GK influences hepatic lipid biosynthesis. The predominant role of this core control system based on β -cell (and perhaps α -cell) and hepatic GK is probably complemented in ways to be explored by the function of other GK-containing cells in the hypothalamus, the brainstem, and the portal vein; in entero-endocrine K- and L-cells; and in gonadotropes and thyrotropes.

Activating GK mutations cause hyperinsulinemic hypoglycemia of a severity that is clearly determined by the degree of GK activation,

including cases with life-threatening seizures, even with only one allele affected. Inactivating mutations cause either the generally mild form of hyperglycemia typical for maturity-onset diabetes of the young (MODY-2) when only one allele is affected or permanent neonatal diabetes resembling type 1 diabetes when both alleles have loss-of-function mutations. Activating GK mutations are located in a circumscribed structural region of GK, as an allosteric modifier region that is clearly separate from the catalytic site. GKAs bind to a distinct activator site in this region involving as contact points many of the amino acids found to be spontaneously mutated in GK-linked hyperinsulinism, whereas GKRPs associate with the enzyme via two amino acid patches in this region with little overlap to the GKA site. Glucose facilitates GKA but inhibits GKRPs binding and action. GKA and GKRPs (glucokinase regulating protein) binding are apparently mutually exclusive. In liver glucose induces a slow conformational transition that dissociates GKRPs/GK but exposes the GKA receptor site. Because islet cells lack GKRPs, their influence on GK/GKA interactions should be ignored. The simplest explanation is that GKA association with GK depends on sugar binding to the catalytic site and that the conformational change, as monitored by tryptophan fluorescence, is the result of cooperative binding of GKA and sugar.

CONCLUSION

GK is the major glucose-sensing enzyme in a number of metabolically responsive organs: brain, liver, gut, and pancreas. In patients with type 2 diabetes, the control of glucose homeostasis is severely disturbed, leading to hyperglycemia. In both type 1 diabetic liver and pancreas, the sensitivity to glucose is reduced. Insulin secretion from diabetic pancreatic islets in response to high glucose is severely impaired, whereas the response to other stimuli can be preserved. In the liver, the ability of high glucose to suppress hepatic glucose production is diminished in diabetic patients. Due to its important role in controlling both glucose-induced insulin secretion and hepatic glucose production, GK is an appealing target for the treatment of type 2 diabetes, and it is postulated that agents enhancing activity of the enzyme should improve glucose control in the disease state. The activators stimulate GK activity via binding to an allosteric site on the enzyme and have been shown to decrease plasma glucose levels in healthy and diabetic animals via stimulation of both insulin secretion and hepatic glucose metabolism. [45]

REFERENCES

1. Dr. AVSS Rama Rao, Enzymes 4th edition. p 160-161, 180-183.
2. U. Satyanarayana, U. Chakrapani, 3rd edition. p 246-247,
3. Cárdenas, M.L., Cornish-Bowden, A., and Ureta, T. Evolution and regulatory role of the hexokinases. *Biochim. Biophys. Acta* 1998; 401: 242-264.
4. Rother KI. Diabetes treatment-bridging the device. *The new England journal of medicine* April 2007; 356(15):499-501.
5. Lawrence JM, Contreras R, Chen W, Sacks DA. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially / ethnically diverse population of pregnant women. *Diabetes care*; 1999-2005; 31(5): 899-904.
6. Cooke DW, Plotnick L (Nov 2008). type 1 diabetes mellitus in pediatrics. *Pediatric Rev* 299(11):374-84.
7. American Diabetes Association: Standards of medical care in diabetes-2006. *Diabetes Care* 29 (Suppl. 1):S4-42, 2006.
8. Cardenas, M.L. (2004). Comparative biochemistry of glucokinase. *Glucokinase And Glycemic Disease: From Basics to Novel Therapeutics (Frontiers in Diabetes)*. Basel: S. Karger AG (Switzerland). 2004; 31-41.
9. Magnuson, M.A. Matschinsky, F.M. Glucokinase as a glucose sensor: past, present, and future. *Glucokinase and Glycemic Disease: From Basics to Novel Therapeutics (Frontiers in Diabetes)*. Basel: S. Karger AG (Switzerland). 2004; 18-30.
10. Iynedjian PB. Molecular physiology of mammalian glucokinase. *Cell. Mol. Life Sci*; 2009; 66 (1): 27-42.
11. Cardenas, M.L. Comparative biochemistry of glucokinase. *Glucokinase and Glycemic Disease: From Basics to Novel Therapeutics (Frontiers in Diabetes)*. Basel: S. Karger AG (Switzerland); 2004: pp. 31-41.
12. Kamata K, Mitsuya M, Nishimura T, Eiki J, Nagata Y. Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. *Structure*. 2004; 12:429-38.
13. Ronimus RS, Morgan HW. Cloning and biochemical characterization of a novel mouse ADP-dependent glucokinase. *Biochem. Biophys. Res. Commun.* ; 2004: 315 (3): 652-8.
14. Magnuson, M.A.; Matschinsky, F.M. . Glucokinase as a glucose sensor: past, present, and future. *Glucokinase and Glycemic Disease: From Basics to Novel Therapeutics (Frontiers in Diabetes)*. Basel: S. Karger AG (Switzerland); 2004: 18-30.
15. Bell, G.I.; Cuesta-Munoz, A.; Matschinsky, F.M. Glucokinase. *Encyclopedia of Molecular Medicine*. Hoboken: John Wiley & Sons; 2002
16. Kamata K, Mitsuya M, Nishimura T, Eiki J, Nagata Y. Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. *Structure*. 2004;12:429-38
17. Matschinsky FM: Assessing the potential of glucokinase activators in diabetes therapy. *Nat Rev Drug Discov*. 2009; 8:399-416. .
18. Agius L: Glucokinase and molecular aspects of liver glycogen metabolism. *Biochem J*. 2008, 414:1-18
19. Del Guerra S, Lupi R, Marselli L, Masini M, Bugliani M, Sbrana S, Torri S, Polera M, Boggi U: Functional and molecular defects of pancreatic islets in type 2 diabetes. *Diabetes*. 2005, 54:727-35.
20. Pal M: Recent advances in glucokinase activators for treatment of type 2 diabetes. *Drug Discovery Today*; 2009, 14:784-92.
21. Coghlan M, Leighton B: Glucokinase activators in diabetes management. *Expert Opin Investig Drugs*; 2008, 17:145-67.
22. Guertin KR, Grimsby J: Small molecule glucokinase activators as glucose lowering agents: a new paradigm for diabetes therapy. *Curr Med Chem*; 2006, 13:1839-43.
23. Sarabu R, Berthel SJ, Kester RF, Tilley JW: Glucokinase activators as new type 2 diabetes therapeutic agents. *Expert Opin Ther Pat*; 2008, 18:759-68.
24. Sarabu R, Grimsby J: Targeting glucokinase activation for the treatment of type 2 diabetes - a status review. *Curr Opin Drug Discov Devel*; 2005, 8:631-7.
25. Matschinsky FM: Banting Lecture. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes*. 1996, 45:223-41.
26. Baltrusch S, Wu C, Okar DA, Tiedge M, Lange AJ: Edited by Matschinsky FM, Magnuson MA, Glucokinase and Glycemic Disease: From Basics to Novel Therapeutics. *Front Diabetes*; 2004, 16:262-74.
27. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of general amber force field. *J Comput Chem*; 2004; 25(9):1157-74.
28. Matschinsky FM: Assessing the potential of glucokinase activators in diabetes therapy. *Nat Rev Drug Discov*; 2009, 8:399-416.
29. Grimsby J, Sarabu R, Corbett WL, Haynes N, Bizzarro FT, Coffey JW, Guertin KR, Hilliard DW, Kester RF, Mahaney PE, Marcus L, Qi L, Spence CL, Teng J, Magnuson MA, Chu CA, Dvorozniak MT, Matschinsky FM, Grippo JF: Allosteric activators of glucokinase: potential role in diabetes therapy. *Science*; 2003, 301:370-3.
30. Pal M: Recent advances in glucokinase activators for treatment of type 2 diabetes. *Drug Discovery Today*; 2009, 14:784-92.
31. Zhi J, Zhai S, Mulligan ME, Grimsby J, Arbet-Engels C, Boldrin M, Balena R: A novel glucokinase activator R04389620 improved fasting and postprandial plasma glucose in type 2 diabetic patients. *Diabetologia*; 2008, 51 (Suppl Suppl 1):S23
32. Bonadonna RC, Kapitza C, Heinse T, Avogaro A, Boldrin M, Grimsby J, Mulligan ME, Arbet-Engels C, Balena R: Glucokinase activator R04389620 improves beta cell function and plasma glucose indexes in patients with type 2 diabetes. *Diabetologia*. 2008, 51 (Suppl Suppl 1):S371.
33. Zhai S, Mulligan ME, Grimsby J, Arbet-Engels C, Boldrin M, Balena R, Zhi J: Phase I assessment of a novel glucose activator R04389620 in healthy male volunteers. *Diabetologia*. 2008, 51 (Suppl Suppl 1):S372
34. Huey R, Morris GM, Olson AJ, Goodsell DS. An empirical free energy force field with charge-based desolvation and intramolecular evaluation. *J Comp Chem*. 2007; 28:1145-1152.

35. Morris GM, Goodsell DS, Halliday RS, et al. Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function. *J Comput Chem*; 1998; 19:1639–62.
36. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc Natl Acad Sci US*; 2001; 98:10037–41.
37. Guesrtin KR, Grimsby J: Small molecule glucokinase activators as glucose lowering agents: a new paradigm for diabetes therapy. *Curr Med Chem*; 2006, 13:1839–43.
38. Sarabu R, Berthel SJ, Kester RF, Tilley JW: Glucokinase activators as new type 2 diabetes therapeutic agents. *Expert Opin Ther Pat*; 2008, 18:759–68.
39. Li C, Liu C, Nissim I, et al. The role of glycine and gamma-hydroxybutyrate in the regulation of glucagon secretion in human islets. 2009 Spring Symposium and Kroc Lecture, University of Pennsylvania, 11 March 2009.
40. Agius L The physiological role of glucokinase binding and translocation in hepatocytes. *Adv Enzyme Regul* 1998; 38:303–331.
41. Agius L Glucokinase and molecular aspects of liver glycogen metabolism. *Biochem J* 2008; 414:1–18.
42. A, Van Schaftingen E. The mechanism by which rat liver glucokinase is inhibited by the regulatory protein. *Eur J Biochem* 1990; 191:483–489.
43. Competitive inhibition of liver glucokinase by its regulatory protein. *Eur J Biochem* 1991; 200:545–551 pmid:188941. Detheux M, . Effectors of the regulatory protein acting on liver glucokinase: a kinetic investigation. *Eur J Biochem*; 1991; 200:553–561.
44. Veiga-da-Cunha M, Van Schaftingen E. Identification of fructose 6-phosphate- and fructose 1-phosphate-binding residues in the regulatory protein of glucokinase. *J Biol Chem* 2002; 277:8466–847.
45. Gromada J, Franklin I, Wollheim CB. “ α -cells of the endocrine pancreas: 35 years of research but the enigma remains.” 2007; 28:84–116.