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REVIEW ARTICLE

GLUCOKINASE ACTIVATORS AS POTENTIAL ANTIDIABETIC AGENTS POSSESSING SUPERIOR GLUCOSE-LOWERING EFFICACY

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ABSTRACT

Currently marketed drugs for type 2 diabetes do not provide adequate glucose control over the long term. Hence, there is still a significant unmet medical need for novel agents that modulate glucose levels with greater and longer-lasting efficacy. Results from several recent studies, including emerging clinical data, have demonstrated that small-molecule glucokinase activators may be able to fill this void. The glucose-sensing enzyme glucokinase plays a pivotal role in whole-body glucose homeostasis through actions in multiple organs, most notably in the liver and pancreas. Glucokinase activators raise the enzyme's activity by increasing its velocity and its affinity for glucose, effects that can result in exquisite glucose lowering when translated into the in vivo setting. Many pharmaceutical companies have initiated drug discovery programs in the area and, in recent years, several organizations have progressed glucokinase activators into clinical trials as potential antidiabetic agents. This review summarizes key pharmacological data associated with several of the leading classes of small-molecule glucokinase activators.

INTRODUCTION: THE NEED FOR NEW TYPE 2 DIABETES THERAPIES

Diabetes is a disease characterized by elevated blood glucose levels, i.e., hyperglycemia. Its prevalence is increasing at an alarming rate, with the number of diabetics worldwide being projected to rise to 366 million in 2030 from 171 million in 2000 (1). At least 90% of diabetics have type 2 diabetes (T2D), a condition strongly associated with aging, sedentary lifestyles and obesity (2). Left untreated, T2D produces microvascular complications (3), e.g., neuropathy, nephropathy and retinopathy, and is associated with an increased risk for cardiovascular disease (4). As a result, this disease has serious implications for both quality and length of life.

T2D is a progressive disorder typified by an early phase of insulin resistance —often associated with obesity— that requires a compensatory increase in insulin secretion from the β-cells of the pancreas to maintain normal blood glucose levels (5). The increased demand for insulin secretion ultimately gives rise to β-cell exhaustion —resulting in defective insulin secretion—, a phenomenon accompanied by elevated levels of hepatic glucose production (Fig. 1). Unfortunately, at present, no single marketed drug can achieve satisfactory, long-lasting blood glucose control in the majority of T2D patients (6). Although combinations of currently available T2D treatments can provide glycemic control superior to monotherapies (7, 8), they are subject to loss of efficacy over time and may also be associated with unwanted side effects. Thus, there is a pressing medical need for safe, novel T2D drugs that reduce glycated hemoglobin (HbA_{1c}) levels —which indicate chronic glucose exposure— more robustly than many of the agents coming to the market (9).

WHY TARGET GLUCOKINASE?

The cytoplasmic enzyme glucokinase (GK) exhibits a fairly restricted expression profile, being localized principally in tissues having a pivotal role in maintaining whole-body glucose homeostasis (10). In particular, this enzyme is expressed in the insulin-secreting β-cells of the pancreas and in the hepatocytes of the liver. However, expression is also seen in other cell types that complete the glucose-sensing network (11), such as the K and L cells of the gut (12), as well as certain neurons of the hypothalamus (13). In addition, it has been reported that GK is expressed in the gonadotropes and thyrotropes of the pituitary gland (14).

GK is the rate-limiting enzyme for glucose uptake and metabolism in both β-cells and hepatocytes. In the pancreas, this enzyme acts as the key molecular sensor that senses the prevailing blood glucose concentration, allowing it to dictate the rate of glucose-dependent insulin secretion accordingly (15). Thus, GK-catalyzed glucose metabolism in the β-cell results in increased adenosine triphosphate (ATP) concentrations via glycolysis. The increased ATP

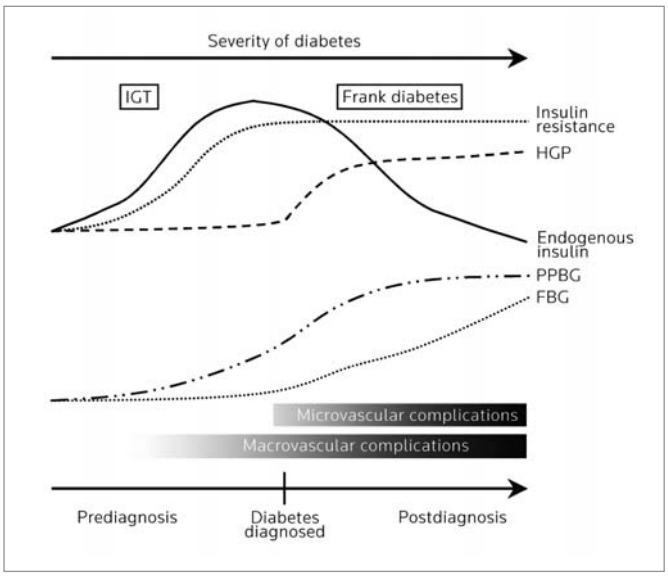

Figure 1. Schematic diagram showing the natural history of type 2 diabetes. The prediabetic state of impaired glucose tolerance (IGT) is characterized by escalating insulin resistance, compensatory hyperinsulinemia and a slight rise in postprandial blood glucose (PPBG) concentrations. Initially, fasting blood glucose (FBG) concentrations remain close to normal. As the disease progresses to frank diabetes, the β-cell starts to fail, resulting in a loss of insulin secretory capacity and higher PPBG levels. Additional reductions in β-cell function result in elevated FBG concentrations accompanied by increased hepatic glucose production (HGP). Adapted from Primary Care, 26(4), Ramlo-Halsted, B.A., Edelman, S.V., *The natural history of type 2 diabetes: Implications for clinical practice*, 771-89, © 1999, with permission from Elsevier.

concentrations lead to closure of ATP-sensitive K^+ channels, membrane depolarization and Ca^{2+} influx, a phenomenon that then triggers insulin release. In the liver, GK removes glucose from the blood through its function as a high-capacity enzyme that catalyzes the reaction of glucose with ATP to form glucose-6-phosphate, which is incorporated ultimately into hepatic glycogen stores (16). Notably, as distinct from the situation in the β-cell, hepatic GK activity is controlled by a regulatory protein (GKRP) (17). In the presence of fructose-6-phosphate (F6P), this regulatory protein inhibits GK competitively and sequesters the enzyme in an inactive state in the nucleus. Following a meal, concentrations of fructose-1-phosphate (F1P) increase, leading to the displacement of F6P, alleviation of the inhibitory actions of GKRP and translocation of active GK to the cytoplasm.

The hexokinase family comprises GK, also referred to as hexokinase-D or hexokinase type IV, and three other homologous isozymes, i.e., hexokinases A, B and C, which have much less restricted expression profiles (18). GK differs from the other hexokinase family members in that it possesses a unique sigmoidal kinetic profile, which makes it perfectly suited to act as a glucose sensor in multiple tissues. Moreover, this enzyme exhibits positive cooperativity with glucose and its half-maximal velocity is observed at a substrate (glucose) concentration ($S_{0.5}$) of about 8 mM, in marked contrast to the other hexokinase isozymes, where much higher affinities for glucose, i.e., lower $S_{0.5}$ values, are noted. Thus, the activity of GK increases rapidly as blood glucose levels rise through the normal physiological range (Fig. 2), i.e., glucose flux through GK soars as blood glucose concentrations increase on going from the fasted (about 5 mM) to the postprandial (about 10 mM) state. This increased flux of glucose through GK leads to augmented insulin secretion from the β-cells and enhanced glucose metabolism in the liver.

The prominent role that GK plays in whole-body glucose homeostasis is underscored by several activating and deactivating mutations found in humans. Individuals with gain-of-function activating GK mutations in a single allele have persistent hyperinsulinemic hypoglycemia, a condition characterized by very low fasting blood glucose levels accompanied by inappropriately high insulin levels (19, 20). This disorder is attributed to a decreased threshold for glucose-stimulated insulin release (GSIR), possibly accompanied by elevated hepatic glucose metabolism. A loss-of-function mutation in one copy of the GK gene, which leads to a GK protein with reduced catalytic activity, leads to a mild form of diabetes called maturity-onset diabetes of the young type 2 (21). Loss-of-function mutations in both GK alleles give rise to a more serious condition known as permanent neonatal diabetes (22), which has to be treated with exogenous insulin. Reduced GK activity has also been implicated in the pathogenesis of T2D: in one study, liver GK activity was decreased by about 50% in obese subjects with T2D (23), while in another, islets from T2D patients had about 40% less GK mRNA than islets from matched nondiabetic donors (24).

GLUCOKINASE ACTIVATORS

Taken together, the GK-linked diseases described above illustrate the importance of GK in whole-body glucose homeostasis and provide a strong justification for targeting agents that treat T2D by raising GK activity. Thus, many pharmaceutical companies have established drug discovery programs looking for small-molecule GK acti-

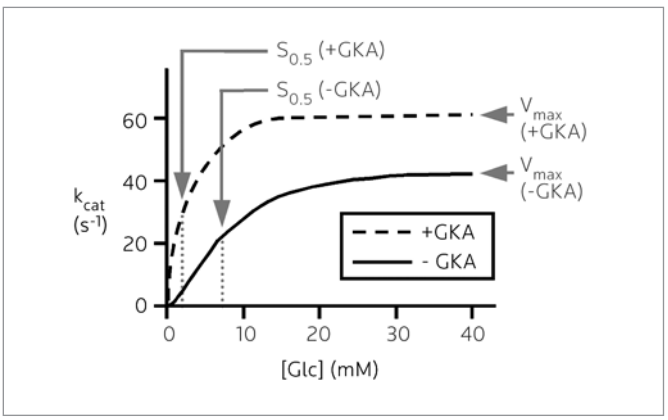

Figure 2. Chart illustrating how glucokinase activators (GKAs) can modify the kinetic profile of glucokinase (GK). In the absence of activator, the glucose (Glc) vs. velocity (k_{cat}) curve of wild-type GK exhibits a sigmoidal response with $S_{0.5}$ being approximately 8 mM. In the presence of the GKA, the Glc vs. k_{cat} curve becomes hyperbolic and the affinity of the enzyme for glucose increases, as evidenced by a lower $S_{0.5}$ value. The particular GKA shown also raises the maximal velocity of GK (V_{max}). All GKAs reported in the literature to date lower $S_{0.5}$ values, but their effects on V_{max} are much more varied.

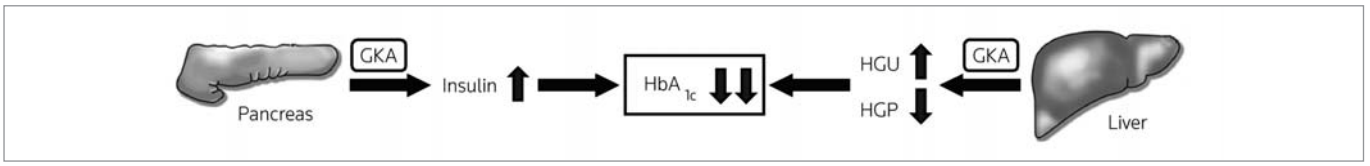

Figure 3. Schematic representation illustrating how glucokinase activators (GKAs) might provide enhanced glycemic control by modifying hepatic glucose balance, by increasing hepatic glucose uptake (HGU) and lowering hepatic glucose production (HGP), and by augmenting pancreatic insulin secretion.

vators (GKAs) (25-31). It is hoped that their efforts will lead to oral antihyperglycemic agents displaying superior, longer-lasting glucose-lowering efficacy as a result of actions in two organs, i.e., the pancreas and the liver (Fig. 3). Indeed, one might surmise that GKAs could provide enhanced glycemic control by combining (32, 33) in a single molecule the glucose-lowering powers of insulin secretagogues, e.g., sulfonylureas, with those of biguanides, e.g., metformin, which modify hepatic glucose balance.

The first GKAs were identified through high-throughput screening efforts designed to look for compounds that could activate GK indirectly by inhibiting GKRP and effecting dissociation of the GK–GKRP complex (34, 35). Although these screens did not unearth drug-like (36) GKRP inhibitors, they did produce small molecules that bind and activate GK.

GKAs can activate GK by enhancing the affinity of the enzyme for glucose, i.e., by decreasing $S_{0.5}$, and/or by raising the turnover of glucose by the enzyme, as evidenced by increased maximal velocity (V_{max}) values (Fig. 2). All GKAs reported in the literature thus far decrease $S_{0.5}$ values, but their effects on V_{max} are much more varied (29, 30, 37). To obtain more efficient catalysis at the elevated blood glucose concentrations characteristic of T2D patients, it is generally considered more desirable to raise V_{max} values (38). GKAs also lower the positive kinetic cooperativity of GK for glucose, driving Hill coefficients down toward unity from a figure of about 1.7 for the free enzyme, and make the characteristic sigmoidal kinetic profile more hyperbolic.

Solid-state structures obtained from GK–GKA cocrystals reveal that the activators bind at an allosteric site on the enzyme some 20 Å remote from, and opposite to, the glucose binding site (Fig. 4). This allosteric activator site is located at the hinge region between the small and the large domains (39-44). Notably, the activating gain-of-function mutations found in individuals with persistent hyperinsulinemic hypoglycemia also map to this site (39). Since the activating mutations co-localize with the allosteric activator site, the activated mutant GK enzymes possess kinetic profiles reminiscent of those produced by GKAs. Thus, all activated mutants have decreased $S_{0.5}$ values, while V_{max} can go up, down or stay the same.

In the solid state, the apo form of GK, i.e., the form not bound to any ligands, exhibits an inactive, "super-open" structure where its large and small domains are widely separated from one another. In contrast, GKA-bound GK adopts a catalytically active, "closed" structure where the two domains of the enzyme come together (Fig. 4). By comparing the structures of the super-open apo form of GK with its GKA-bound counterpart, a model that rationalizes the positive cooperativity of GK with respect to glucose was formulated by Kamata et al. (40). In this model (Fig. 5), GK can exist in one of three conformations, i.e., the closed and super-open forms, as well as a state intermediate between these two forms, termed the "open form". Interconversion between these three conformations occurs via two catalytic cycles, i.e., a "fast" cycle, which bypasses the lower-energy super-open form, and a "slow" cycle, which requires more substantial conformational rearrangements because of the involvement of the super-open form. The ratio of the two catalytic cycles is dependent on the prevailing glucose concentration and is responsible for GK's unique sigmoidal kinetic profile. Under low glucose conditions, the thermodynamically more stable super-open form predominates and catalysis proceeds primarily through the slow cycle. As glucose concentrations rise, the enzyme switches catalytic cycles such that the fast cycle is employed to a greater extent.

The allosteric activator binding pocket is not present in the super-open state, but it is seen in both the open and closed states. As a result, when GKAs bind to this hydrophobic pocket, the enzyme cannot relax back to the lower-energy super-open state, which has a lower affinity for glucose. Instead, the enzyme can only exist in the high-affinity open or closed states, locking it into the fast catalytic cycle even at low glucose concentrations. In effect, GKAs, like activating GK mutations, stabilize the open and closed forms of the

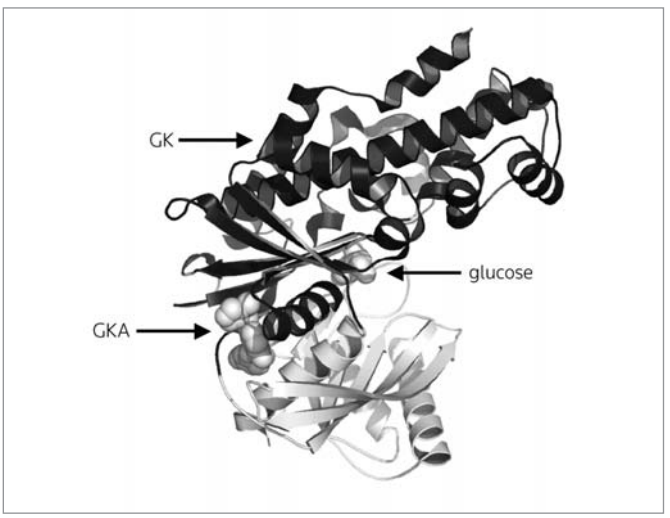

Figure 4. Ribbon drawing of a glucokinase activator–glucokinase–glucose (GKA·GK·glucose) cocrystal structure highlighting the allosteric GKA binding site and the glucose binding site.

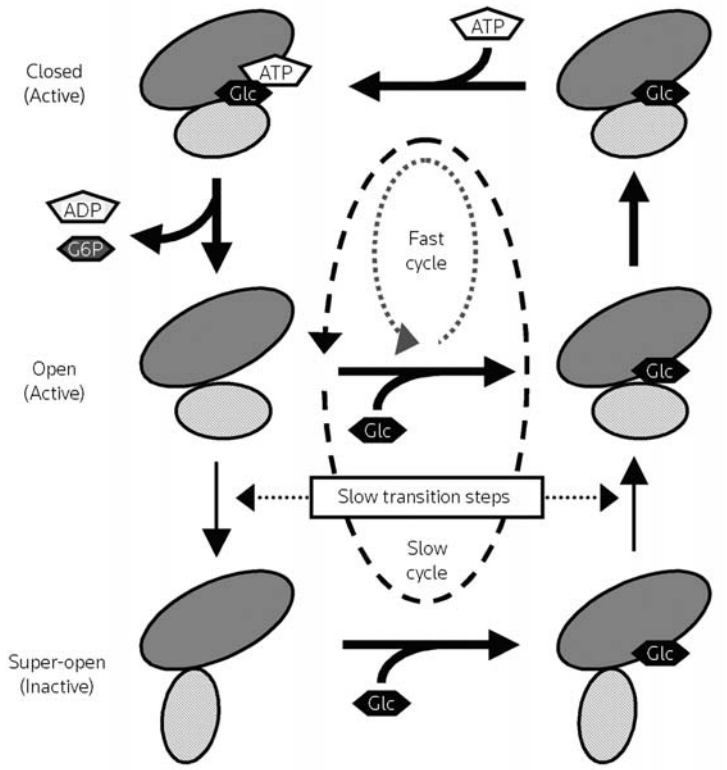

Figure 5. Kamata's model describing how glucokinase (GK) catalyzes the reaction of glucose (Glc) with adenosine triphosphate (ATP) to generate glucose-6-phosphate (G6P) and adenosine diphosphate (ADP). GK appears to exist in three conformations associated with two catalytic cycles. The ratio of these two catalytic cycles is responsible for the sigmoidal kinetic response to Glc. Low Glc concentrations favor the "super-open", low-affinity conformation, resulting in catalysis through the slow catalytic cycle. In contrast, high Glc concentrations favor the "open" and "closed", high-affinity conformations associated with the fast catalytic cycle. GKAs stabilize these high-affinity conformations by binding to the allosteric pocket found at the hinge region between the two lobes of the enzyme and increase GK's affinity for Glc in the process. Adapted from Structure, 12(3), Kamata, K., Mitsuya, M., Nishimura, T., Eiki, J., Nagata, Y., *Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase*, 429-38, © 2004, with permission from Elsevier.

enzyme by reducing the exposure of the hydrophobic, allosteric activator binding pocket to solvent (39). Notably, the allosteric activator site is not present in the other hexokinase isozymes, which exist only in closed or open forms. As a result, GKAs have no effects at any of the other hexokinases. Tryptophan fluorescence studies have recently confirmed the existence of multiple GK conformations in solution (45).

KEY GLUCOKINASE ACTIVATORS

As indicated above, numerous pharmaceutical companies have initiated drug discovery programs seeking to discover novel, orally available GKAs that could form the basis of new T2D therapies. The extensive patent literature emanating from these programs has been reviewed elsewhere (28, 46) and will not be discussed here. Instead, this review will assess the potential of GKAs as potent glucose-lowering drugs by considering data from pharmacological studies with key GKAs.

RO-28-1675

The archetypal GKA is Roche's **RO-28-1675** (Fig. 6), a compound first described in a seminal paper by Grimsby et al. (47). In vitro, at a concentration of 3 μM, RO-28-1675 increased V_{max} by about 1.5-fold and decreased $S_{0.5}$ from 8.6 mM to 2.0 mM. Furthermore, this GKA reversed the inhibitory actions of GKRP. In freshly isolated perifused rat pancreatic islets, which contain the insulin-secreting β-cells, RO-28-1675 shifted the GSIR curve to the left, i.e., it triggered insulin release at lower glucose concentrations. Moreover, islets that had been cultured with RO-28-1675 at basal glucose levels displayed, after compound washout, an increased maximal response to GSIR, comparable to that observed in islets cultured under high glucose in the compound's absence.

Following acute oral administration in vivo, RO-28-1675 displayed potent glucose-lowering actions (38, 47). In nondiabetic rodents, these glucose-lowering actions resulted in lower than normal blood glucose levels, i.e., hypoglycemia. In contrast, in diabetic rodent models, treatment with RO-28-1675 normalized blood glucose con-

centrations. The blood glucose-lowering effect of this compound was associated with increased insulin concentrations. RO-28-1675 also attenuated postprandial glucose excursions in both nondiabetic and diabetic rodents, as evidenced by reduced glucose exposures in oral glucose tolerance tests (OGTTs). In pancreatic clamp studies, it was shown that this compound reduced hepatic glucose production and enhanced hepatic glycogen synthesis (48). In some cases, RO-28-1675 even reversed hepatic glucose production such that the liver became a net importer of glucose. RO-28-1675 did not exhibit tachyphylaxis in a chronic 40-week study in diet-induced obese (DIO) mice. In this study, mice placed on a high-fat diet (HFD) supplemented with RO-28-1675 did not develop hyperglycemia, unlike their counterparts receiving the HFD only, although body weights were similar in both groups.

RO-28-1675 was evaluated in the clinic in healthy male volunteers (49, 50). At 25 mg, this GKA did not produce any effects on fasting blood glucose levels. However, at 200 and 400 mg it significantly attenuated the glucose excursion in an OGTT. The only side effect noted in this study was hypoglycemia at the higher dose.

The development of RO-28-1675 was halted as a result of an unacceptable cardiovascular risk profile (51) and the formation of a potentially troublesome thiourea metabolite (52).

Piragliatin

Piragliatin (Fig. 6) is the GKA for which the most human pharmacokinetic and pharmacodynamic data are available. Like RO-28-1675, this compound originated at Roche (52) and is also known as RO-4389620, R-1440 and GK2. It completed phase II clinical trials, but development was terminated for undisclosed reasons and it was replaced in the Roche pipeline by a prioritized backup compound called R-1511 (29).

In a single-ascending-dose study in healthy male volunteers, piragliatin was absorbed rapidly and displayed an elimination half-life of 8-10 h (53). This compound exhibited a dose-proportional pharmacokinetic profile from 5 to 25 mg, a feature that was associated with dose-dependent reductions in fasting plasma glucose levels. It was also well tolerated up to 25 mg, the most common adverse event being headache.

In a separate study, the effects of piragliatin on β-cell function, endogenous glucose output and glucose utilization in T2D patients were evaluated at doses of 25 and 100 mg (54, 55). This GKA effected dose-dependent reductions in fasting plasma glucose levels, which were associated with augmented insulin secretion —as evidenced by increasing C-peptide concentrations— as well as with reduced endogenous glucose production and a slight increase in glucose utilization. Moreover, piragliatin improved glucose tolerance dose-dependently in an ensuing OGTT, a phenomenon that could be ascribed to higher glucose utilization in the period following administration of the glucose load. An analysis of the OGTT plasma glucose and C-peptide concentration curves revealed that piragliatin increased the sensitivity of the β-cells both to the rate of increase of glucose and to the glucose concentration itself, i.e., it improved β-cell function. As a cautionary note, however, at the highest dose, it was necessary to administer intravenous "rescue" glucose to overcome unwanted hypoglycemic effects.

In a subsequent multiple-ascending-dose study over 6 days in T2D patients, piragliatin dose-dependently decreased fasting plasma glucose and improved postprandial glucose excursions (56). The only dose-limiting adverse events in this study involved mild or moderate hypoglycemic episodes, which could be overcome by sugar-containing drinks or meals.

Researchers from Pfizer have recently reported analogues of RO-28-1675 and piragliatin, e.g., **1** (Fig. 6), where the 4-methanesulfonylbenzene moiety is replaced with a 4-methanesulfonyl-2-pyridone group (57).

GKA-50 and related GKAs

In the past 5 years, AstraZeneca researchers have published various results from their GKA program. This program has furnished two GKAs, AZD-6370 and AZD-1656, which, at the time of writing, were in phase II clinical trials as potential T2D therapies. Unfortunately, however, at present, no data on these compounds have been made publicly available.

The first AstraZeneca GKA publications centered on **GKA-1** and **GKA-2** (Fig. 7), compounds which increased the affinity of GK for glucose by 4- and 11-fold, respectively, at 10 μM (34). In contrast to RO-28-1675 (vide supra), neither of these compounds altered V_{max} values. In hepatocytes, they both stimulated glucose phosphorylation, glycolysis and glycogen synthesis by a mechanism independent of GKRP. Moreover, they brought about translocation of GK from the nucleus to the cytoplasm, despite the fact that they did not dissociate GK from GKRP. GKA-1 dose-dependently decreased blood glucose levels in overnight-fasted female mice (58).

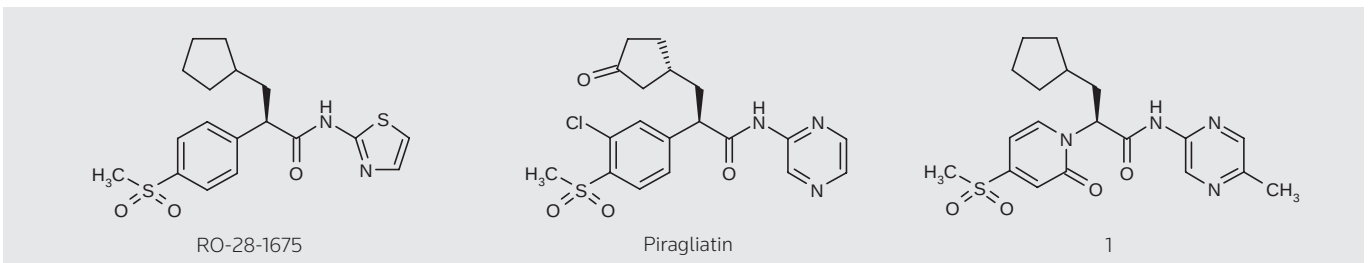

Figure 6. Chemical structures of Roche's glucokinase activators (GKAs) RO-28-1675 and piragliatin, as well as the related GKA **1**.

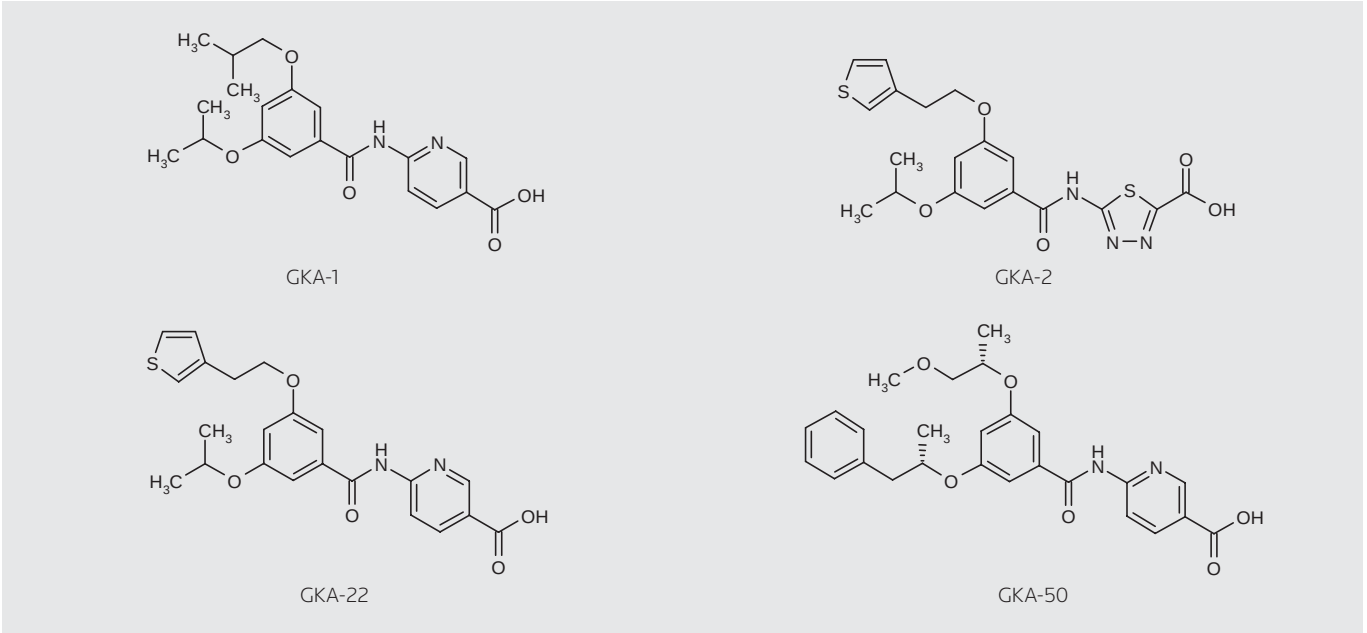

Figure 7. Chemical structures of glucokinase activators (GKAs) from AstraZeneca.

Optimization of GKA-1 furnished **GKA-22** (59) and **GKA-50** (60) (Fig. 7), compounds deemed to possess a suitable balance of potency and physical properties for further in vivo analyses. GKA-50 in particular was extremely potent in vivo, reducing the glucose excursion in an acute OGTT in female HFD-fed Zucker rats at oral doses as low as 1 mg/kg. The blood glucose-lowering effect elicited by GKA-50 and other related compounds was used to derive a pharmacokinetic–pharmacodynamic relationship linking the free, non-protein-bound GKA concentrations to significant blood glucose lowering in vivo (61). This relationship indicates that significant blood glucose lowering is observed at unbound GKA-50 concentrations greater than the potency obtained from in vitro GK activation measurements. GKA-50 has also been shown (62-65) to raise intracellular Ca^{2+} concentrations and stimulate insulin secretion from rodent and human islets, as well as from various β-cell lines. Notably, in contrast to the sulfonylurea tolbutamide, GKA-50 failed to elevate Ca^{2+} concentrations and initiate insulin release at low glucose concentrations (0-2 mM) in rat islets (62, 63).

Much data have also been proffered on another AstraZeneca GKA, GKA-71 (66), for which the molecular structure is unknown. Following chronic culture of rodent islets in the presence of GKA-71 and glucose, the expression of key β-cell genes, including insulin (*INS*) and glucose transporter type *2* (*GLUT2*), was increased (67). This outcome indicates that GKAs may possess chronic β-cell-preserving characteristics (68) in addition to their ability to improve blood glucose homeostasis acutely. GKA-71 also improved glucose control subchronically in obese male Zucker rats and significantly decreased HbA_{1c} levels (69). In a 4-week study in insulin-resistant, HFD-fed mice (70), GKA-71 reduced basal plasma glucose concentrations to normal levels. Furthermore, this compound improved glucose tolerance to the same extent in intravenous glucose tolerance tests (IVGTTs) carried out after 1 and 4 weeks. Insulin levels were actually reduced during the IVGTTs, a result that could be ascribed to improved insulin sensitivity. Notably, at the end of this 4-week study, no significant differences in liver glycogen and triglyceride levels were observed between GKA-71- and control-treated mice. This is important because, following experiments with transgenic animals, concerns have been raised that the activation of hepatic GK could give rise to accumulation of glycogen and lipid in the liver (71). However, these concerns have been allayed by the fact that, following chronic administration of GKAs in preclinical models, no adverse effects on liver glycogen or lipid levels have been noted (30).

GKAs from Merck & Co.

The GKA literature has been enriched considerably through multiple important contributions from the group at Merck & Co.'s Banyu Tsukuba Research Institute. Of particular note in this regard, following elegant crystallographic studies, Merck researchers reported that the GKA **2** (Fig. 8) activated GK by binding to an allosteric site and formulated the model (Fig. 5) accounting for the cooperativity of the enzyme with respect to glucose (40). As expected, the related GKA **3** also binds to the allosteric site (43) and, like **2**, activates GK by raising V_{max} and lowering $S_{0.5}$ (72, 73). Compound **3** stimulated insulin secretion from isolated rat islets and enhanced glucose metabolism in primary cultured rat hepatocytes. In hepatocytes, this compound also reduced glucose output following a dihydroxyacetone challenge. The combined pancreatic and hepatic actions of **3** gave rise to significant blood glucose lowering in a number of rodent models. Notably, in both isolated rat primary hepatocytes and liver tissues, **3** caused the translocation of GK from the nucleus to the

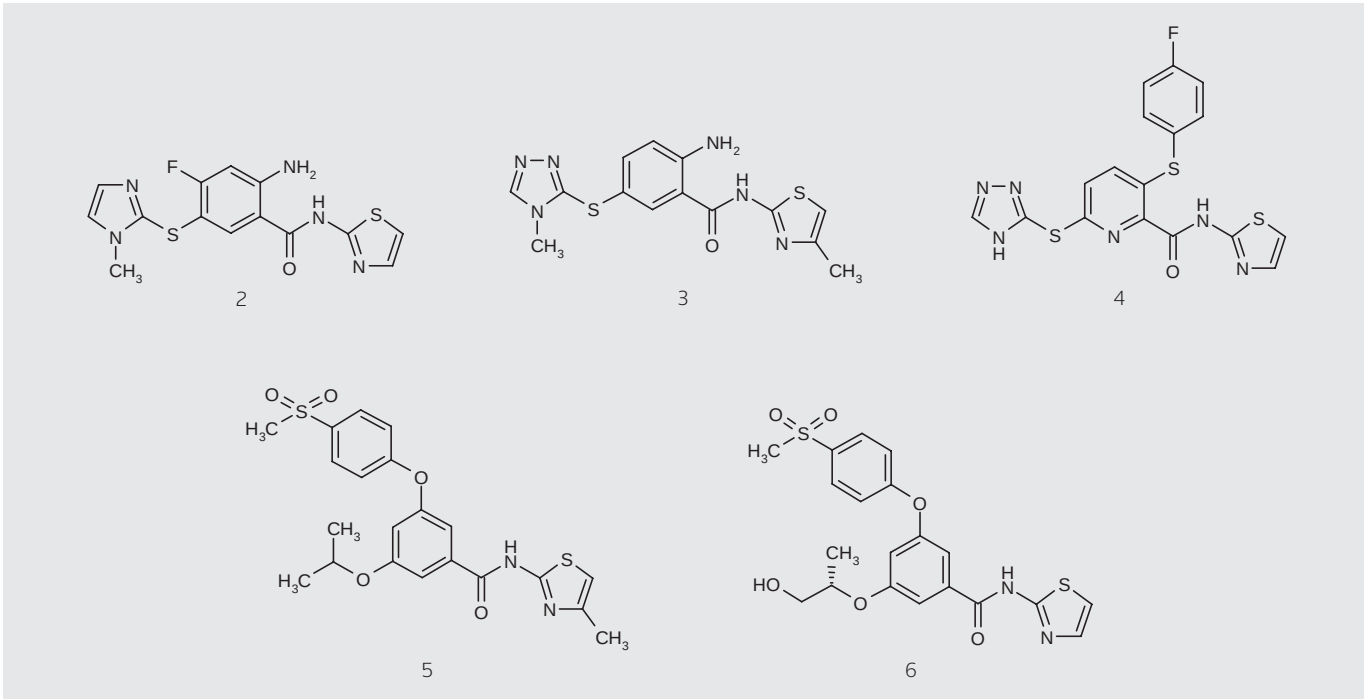

Figure 8. Chemical structures of glucokinase activators (GKAs) from Merck & Co.

cytoplasm, an event that was reversible upon compound removal. The translocation event was attributed to the GKA stabilizing the activated glucose-bound state of GK, which resulted in an impaired interaction between the enzyme and its regulatory protein. Interestingly, it was demonstrated that **3** interacts only with glucose-bound GK and not with GKRP-associated GK or the free enzyme when it is not bound to a glucose molecule.

The effects of a related GKA on hepatic glucose uptake were evaluated in a conscious dog model of hyperglycemia induced by constant intraportal glucose infusion (74). In this model, although there was an absence of insulin secretion —because of a somatostatin infusion— orally administered GKA still increased net hepatic glucose uptake, indicating direct effects on the liver during postprandial hyperglycemia. The hepatic actions of a Merck GKA were also demonstrated in a pancreatic hyperinsulinemic–hyperglycemic clamp study in rats (75). In this study, the GKA increased hepatic glucose uptake and glycogen synthesis while suppressing endogenous glucose production.

Another Merck GKA was reported to improve glucose metabolism in mice with a β-cell-specific haploinsufficiency of the GK gene (i.e., *Gck*$^{+/-}$) fed a HFD (76). These same *Gck*$^{+/-}$ mice were utilized in studies investigating the effects of an analogue of **2** on β-cell proliferation and mass (77, 78). Although the GKA did not increase β-cell mass in vivo after 20 weeks of treatment, it did increase β-cell proliferation in vitro and also in vivo following a shorter 3-day treatment. Interestingly, GKA treatment upregulated the expression of insulin receptor substrate 2 (IRS-2), a key mediator of β-cell growth and survival (79), in insulinoma cells and isolated islets. The apparent discrepancy between the in vitro and the chronic in vivo results was attributed to the sustained reduction in the prevailing blood glucose concentrations by the GKA in a chronic setting. This reduction leads to a lowering of the hyperglycemia-associated stimuli that provoke an increase in islet mass.

Merck researchers have also performed initial studies (80) aimed at differentiating GKAs from the widely prescribed sulfonylureas (81), antidiabetic drugs with less durable efficacy than other therapies (82). In an acute setting, the sulfonylurea glimepiride and the GKA under evaluation reduced glucose levels to a similar extent, although insulin levels were increased only in the sulfonylurea-treated rats. However, in contrast to the GKA, where efficacy was retained until the end of the 35-day study, glimepiride lost its efficacy within 3 days. Moreover, the GKA retained its full glucose-lowering efficacy in rats that had been desensitized to sulfonylureas following treatment with glimepiride for 14 days, unlike another sulfonylurea, glipizide, which barely elicited any glucose lowering. These data suggest that GKAs may be able to elicit their antihyperglycemic actions in patients who are no longer responsive to sulfonylurea therapy. In a separate study, contrary to the sulfonylurea glipizide, a Merck GKA prevented hydrogen peroxide-induced acute cell death when incubated with MIN6 clonal mouse pancreatic β-cells (83). The same compound also protected MIN6 cells from apoptosis induced by glucose deprivation, a finding associated with enhanced cellular energy metabolism and increased association of GK with mitochondria.

Details of the optimization of GKAs at Merck have been published recently. In particular, studies aimed at removing the undesirable aniline moiety of **3** have led to **4** (44) and **5** (84). The GKA **5** has subsequently been optimized to **6** (Fig. 8), a compound with improved

aqueous solubility and significant blood glucose-lowering efficacy in HFD-fed and KKA^y mice (85).

Merck recently reported data from a phase I study in nondiabetic subjects with the GKA MK-0599 (86). This compound exhibited a half-life of approximately 12 h and it dose-dependently lowered weighted mean glucose concentrations at doses of 13-100 mg given three times daily. At the time of writing, MK-0599 is not included in Merck's product pipeline and does not appear to be under active development.

PSN-GK1 and related GKAs

The potent GKA **PSN-GK1** (Fig. 9) was obtained by researchers from OSI Prosidion and Tanabe (87) following optimization of the high-throughput screening hit **7**. PSN-GK1 activated GK primarily by reducing glucose $S_{0.5}$ values (88), although an increase in V_{max} was also noted. This compound stimulated insulin secretion at 5-15 mM glucose in the MIN6 β-cell line, and it also increased the uptake of the tritiated nonmetabolizable glucose analogue 2-deoxyglucose in cultured rat hepatocytes. In nondiabetic mice, the pancreatic effects of PSN-GK1 were evidenced by increased insulin secretion, together with potent glucose lowering. The hepatic actions of PSN-GK1 were confirmed in a hyperinsulinemic–hyperglycemic clamp study in mice, where the liver glycogen biosynthesis rate was significantly increased. Moreover, PSN-GK1 also exhibited potent antihyperglycemic actions in rodent models of diabetes, in both acute and subchronic settings. Notably, in the subchronic arena, blood glucose lowering was achieved without concomitant alterations in lipid levels, liver weight, glycogen content or body weight.

The OSI Prosidion and Tanabe team also published research (89) leading to the discovery of urea **8** (Fig. 9), a GKA with relatively low potency compared to PSN-GK1. This compound lowered blood glucose levels in mice, but at doses substantially higher than those at which PSN-GK1 exhibited significant antihyperglycemic effects.

In rats, the OSI Prosidion GKAs PSN-105 and PSN-010 displayed longer- and shorter-acting pharmacokinetic profiles, respectively, despite activating GK to a similar extent (90). Although both compounds attenuated the glucose excursion similarly in acute OGTTs in *ob*/*ob* mice, PSN-105 exhibited a longer-lasting antihyperglycemic effect that was evident long after the glucose spike. OSI Prosidion's GKA program, including clinical candidate PSN-010, was licensed to Lilly in January 2007 (91).

LY-2121260

Before in-licensing PSN-010, Lilly had its own GKA program (41, 92), which furnished the cyclopropyl-containing GKA **LY-2121260** (Fig. 9). Like other GKAs, this compound binds to the allosteric activator site located in the hinge region of the enzyme. It stimulated insulin secretion glucose-dependently in β-cells and increased glucose utilization in rat hepatocytes. The combined pancreatic and hepatic actions of this compound led to the expected blood glucose lowering in OGTTs in male Wistar rats. Interestingly, GK protein levels rose following incubation of β-cells with LY-2121260, a phenomenon that implies that the enzyme adopts a more stable conformation following binding to activator ligands. Hence, enhanced GK activity resulting from chronic treatment of β-cells with GKAs may be ascribed not only to modified enzyme kinetics, but also to increased enzyme quantities.

TTP-355

In 2007, TransTech Pharma reacquired all rights to a GKA program that it had previously partnered with Novo Nordisk (93). The compounds from this program are reported to normalize blood glucose levels without causing hypoglycemia as a result of liver-selective activation of GK, a phenomenon that has been demonstrated with the clinical candidate TTP-355 (94). Thus, while this compound significantly increased glucose metabolism in both rat and human hepatocytes, it did not induce insulin release from isolated mouse islets, perfused rat pancreata or the rat insulinoma β-cell line

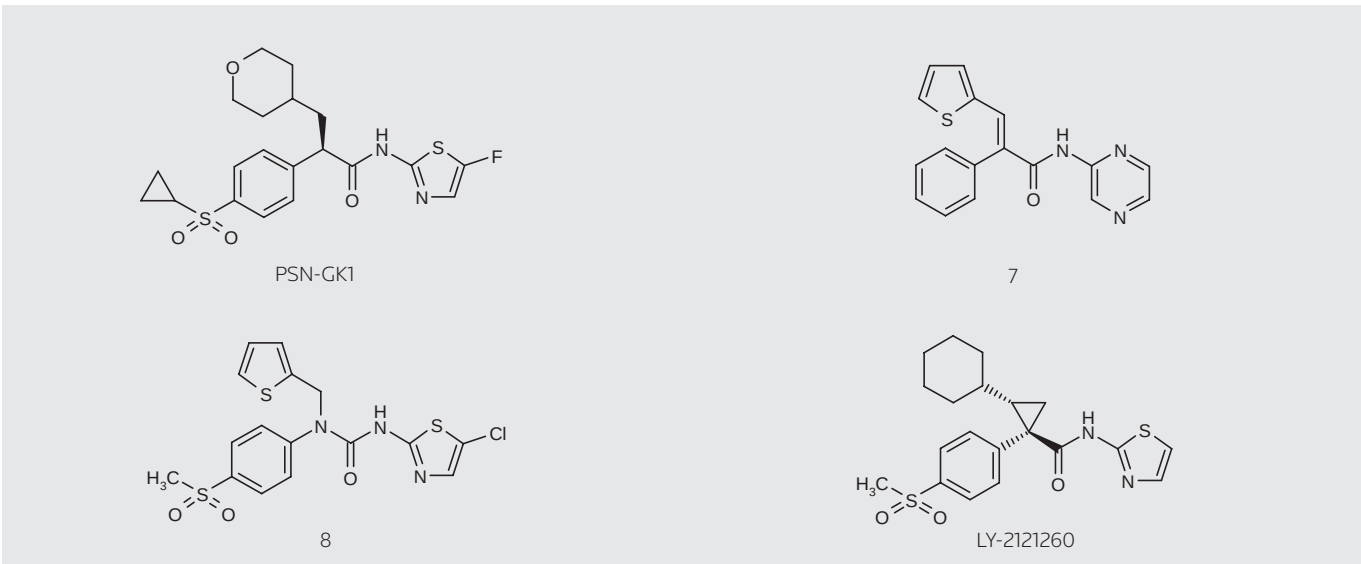

Figure 9. Chemical structures of glucokinase activators (GKAs) from OSI Prosidion and Lilly.

INS-1E. Furthermore, TTP-355 elicited a dose-dependent attenuation of the glucose excursion in rat OGTTs without increasing insulin levels (95). This compound also lowered HbA_{1c} levels substantially in subchronic studies in mildly diabetic Goto Kakizaki rats and in diabetic *ob*/*ob* mice. Interestingly, there was an improvement in β-cell morphology, which was accompanied by a trend towards increased β-cell mass, in the Goto Kakizaki rat study. In type 1 streptozotocin-diabetic minipigs, chronic treatment with TTP-355 lowered the amount of exogenous insulin required to maintain glucose control, moderated the daily glucose excursions in response to a meal and increased glucagon-like peptide 1 (GLP-1) secretion. Notably, TTP-355 did not induce hypoglycemia when administered at high dose levels to fed or fasted, normal or diabetic, animals.

TTP-399 is a backup compound to TTP-355. This compound represents a different chemical series and is currently in preclinical development.

ARRY-403 and related GKAs

In contrast to the other GKAs described above, the efforts of the Array BioPharma group have focused on nonamide GKAs (96, 97) (Fig. 10). Optimization of a novel lead furnished ARRY-588, a GKA that dose-dependently attenuated glucose excursions in OGTTs on days 1 and 14 of a subchronic study in diabetic *ob*/*ob* mice and reduced fasting glucose to levels comparable to those seen in nondiabetic C57BL/6J mice (97). The blood glucose lowering elicited by ARRY-588 was accompanied by reductions in serum lipids and insulin at the end of the study, and no effects on body weight were seen. The pharmacokinetic profiles of ARRY-588 in higher species, however, were not good enough to warrant its further development.

As a consequence, ARRY-588 was further optimized to afford ARRY-403 (98), a GKA that Array subsequently advanced into phase I clinical trials (99). It is claimed that ARRY-403 has a lower hypoglycemic risk than other GKAs (98, 100). Thus, in OGTTs in nondiabetic C57BL/6J mice, while ARRY-403 showed a dose–response relationship for glucose lowering following oral administration at 3 and 10 mg/kg, little further attenuation of the glucose excursion, beyond that seen at 10 mg/kg, was noted at 30 mg/kg. In addition, while ARRY-403 produced significant glucose lowering in female ZDF rats, it did not decrease glucose levels into the "hypoglycemic" 3-4 mM range, unlike PSN-GK1. The reduced hypoglycemic potential of ARRY-403 has been ascribed to the fact that this compound reduces the glucose $S_{0.5}$ to about 1 mM at a concentration of 5 μM, in contrast to other GKAs, which lower $S_{0.5}$ to an even greater extent. In a subchronic study in diabetic *ob*/*ob* mice, ARRY-403 controlled both fasting and post-challenge glucose levels (101). At higher dose levels, ARRY-403's blood glucose-lowering effect was substantially better than that of a maximally effective dose of the marketed dipeptidyl peptidase 4 (DPP IV) inhibitor sitagliptin (102). Moreover, this compound's efficacy in OGTTs appeared to be enhanced upon multiple dosing compared to the effects seen following a single dose. In a combination study in HFD-fed female ZDF rats (103), sitagliptin enhanced the ability of ARRY-403 to attenuate the glucose excursion in an OGTT. Likewise, the glucose-lowering properties of ARRY-403 in HFD-fed female ZDF rats were also enhanced by combination with the antidiabetic drugs metformin and pioglitazone. Notably, in contrast to pioglitazone, an antidiabetic agent that increases body weight in both rats (104) and humans, ARRY-403 was weight-neutral in these studies.

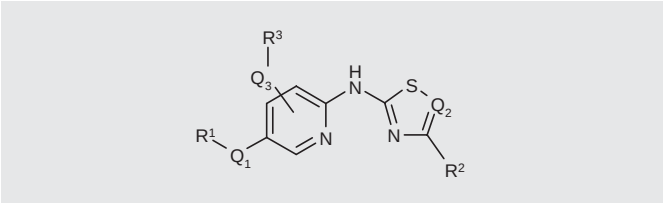

Figure 10. Novel glucokinase activator (GKA) core structures identified by Array BioPharma that lack the central amide bond observed in other GKAs.

CONCLUSIONS

Agents that lower glucose levels more effectively and more durably than existing oral antidiabetic drugs represent an important unmet need (9). In contrast to established antidiabetic therapies with a single site of action, such as sulfonylureas, GKAs represent an appealing opportunity to address this unmet medical need since they exert their effects through multiple organs. However, the exquisite blood glucose-lowering potential of GKAs comes at a price, in the form of a possible hypoglycemic risk. Coghlan and Leighton (30) have proposed that the hypoglycemic risk of GKAs could be substantially lower in T2D patients than in the nondiabetic population. In their proposal, GKAs simply "reset the GK glucostat", regulating GK activities and glucose levels without causing hypoglycemia (Fig. 11). This proposal is supported by preclinical studies with GKAs in diabetic animal models (38, 88), where blood glucose levels are normalized without hypoglycemia, in contrast to the situation in nondiabetic animals, where glucose concentrations fall from normal levels into the hypoglycemic range. Regardless of whether this proposal holds true, as a result of the fact that their glucose lowering is glucose-dependent (15, 105), GKAs should have a lower hypoglycemic potential than sulfonylureas, drugs that elicit their glucose-lowering effect via glucose-independent mechanisms (106).

Sulfonylureas represent a class of oral antidiabetic agents that GKAs would probably seek to replace. Hence, it is imperative that GKAs differentiate themselves from these agents, which are relatively inexpensive and provide robust HbA_{1c} lowering in T2D patients (81). In this regard, it is noteworthy that, unlike sulfonylureas, GKAs do not initiate insulin release at low glucose concentrations (62, 63), an indicator of hypoglycemic potential, and do not appear to lose their efficacy upon chronic administration (80), i.e., they may exhibit superior long-term glucose control. Moreover, while sulfonylureas appear to accelerate β-cell apoptosis and exhaustion (81, 107), preliminary results suggest that GKAs could possess β-cell-protective actions (67, 77, 78, 83). In addition, while sulfonylureas tend to increase body weight (81), in all chronic studies reported to date GKAs have been weight-neutral (38, 88, 103). Indeed, it has been postulated (108) that GKAs could even reduce body weight and the AstraZeneca GKA AZD-1656 is purported to be in the clinic for both diabetes and obesity.

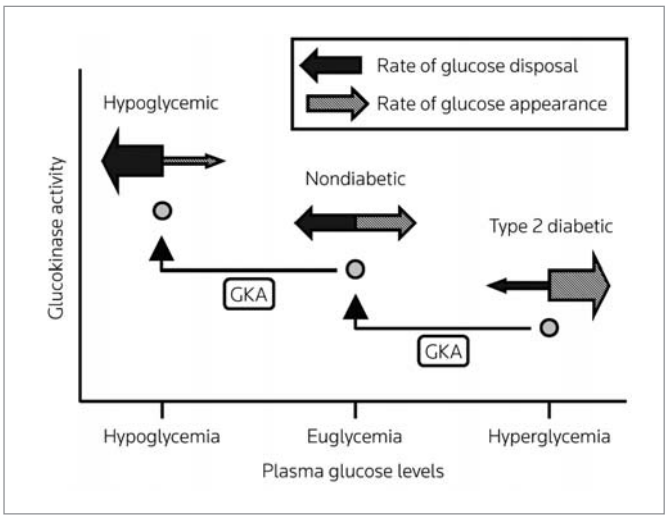

Figure 11. Coghlan and Leighton's model wherein glucokinase activators (GKAs) "reset the GK glucostat" and normalize blood glucose levels in T2D patients, but lower glucose concentrations into the hypoglycemic range in normal subjects. In diabetics, GKAs restore GK activity to normal levels, thereby reducing the rate of glucose appearance, the sum of exogenous glucose from food and endogenous glucose output from the liver, and increasing the rate of glucose disposal into the liver and muscle. In nondiabetic individuals with intact glucose homeostasis and normal GK activity, GKAs may excessively reduce the rate of glucose appearance and inordinately raise the rate of glucose disposal, leading to hypoglycemia. Adapted by permission from Informa Healthcare: Expert Opin Investig Drugs, Coghlan, M., Leighton, B. *Glucokinase activators in diabetes management*, 17(2): 145-67, © 2008.

Nevertheless, although GKAs have demonstrated the potential to have effects beyond blood glucose lowering in various studies, some organizations do view the potential therapeutic benefits of GKAs as being limited. In this regard, Roche recently reported that it had decided to discontinue all development of early-stage compounds that would "only lower glucose levels", including all GKAs (109).

In conclusion, preclinical studies in rodents have demonstrated that GKAs have the potential to be potent antihyperglycemic agents with enduring effects and added benefits beyond glucose lowering alone. As a result, several of these compounds have entered human clinical trials and initial indications are that the glucose lowering observed in rodents is maintained in a human setting, at least in the short term. Results from longer-term clinical trials are eagerly awaited and will determine whether GKAs can form the basis of next-generation antidiabetic therapies.

DISCLOSURE/ACKNOWLEDGMENTS

M.C.T. Fyfe and M.J. Procter are employees of OSI Prosidion and own stock and have stock options in OSI Pharmaceuticals. C. Sambrook Smith is acknowledged for his assistance in preparing Figure 4.

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