Glucokinase Activators for Diabetes Therapy

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Type 2 diabetes is characterized by elevated blood glucose levels resulting from a pancreatic β-cell secretory insufficiency combined with insulin resistance, most significantly manifested in skeletal muscle and liver (1). If untreated, diabetic complications develop that cause loss of vision, peripheral neuropathy, impaired kidney function, heart disease, and stroke. The disease has a polygenic basis because numerous genes (the latest count exceeding 20) participate in its pathogenesis, but modern lifestyle characterized by limited physical activity and excessive caloric intake are critical precipitating factors for the current epidemic of type 2 diabetes worldwide (2). It appears that available treatments, including attempts at lifestyle alterations and drug therapies including insulin, are insufficient to stem the tide. Therefore, new approaches, including the development of therapeutic agents with novel mechanisms of action, are needed.

Selection of new drug targets to treat type 2 diabetes has to be guided primarily by consideration of established physiological chemistry of glucose homeostasis and of prevailing views about the pathophysiology of type 2 diabetes because the genetics of the disease that could serve as another guiding principle remain prohibitively perplexing. The glucose-phosphorylating enzyme glucokinase (GK) was identified as an outstanding drug target for developing antidiabetic 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 and glycogen synthesis, both processes that are impaired in type 2 diabetes (3). Milestones in the 45-year history of GK research are listed in Supplementary Table 1 (Supplementary References S1–S27). In the mid-1990s, Hoffmann La-Roche scientists conducted a high-throughput screen in search of small molecules that could reverse the inhibition of GK by its regulatory protein (GKRP, see further discussion below) and identified a hit molecule that reversed GKRP inhibition by directly stimulating GK (4). Lead optimization efforts resulted in the discovery of several potent preclinically effective and apparently safe compounds and culminated in the development of piragliatin (for example, see structures in Supplementary Fig. 1), which was advanced through phase 2 trials but was then discontinued for undisclosed reasons. Since the first 2003 report in *Science* (4) on the discovery and preclinical effects of GK activators (GKAs), >100 patents for GKAs, several publications, and disclosure of early studies in type 2 diabetes have contributed significantly to the proof of principle and keen interest of pharmaceutical industry for this class of new antidiabetic agents (5–13). In the following contribution, the essential aspect of the role of GK in glucose homeostasis, of the biochemical pharmacology of GKA, and of the current status of GKA research and development and therapeutic application are discussed.

ROLE OF GK IN GLUCOSE HOMEOSTASIS AS CURRENTLY UNDERSTOOD—To comprehend the mechanism of action of GKAs and the rationale for using them as antidiabetic agents, the role of GK at the molecular, cellular, organ, and whole-body level has to be understood (3,14). GK is unique among hexokinase isoenzymes. 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 (has a Hill slope of 1.7) 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" conformation (15–17). The binary complex with glucose has not been crystallized, but fluorescence studies using tryptophan suggest that the structure is very similar to that of the ternary complex (18). There is strong evidence that the transition between super-open and closed forms is slow compared with the rate of the catalytic cycle that is used to explain the physiologically important cooperativity with regard to glucose (19,20; see below for further discussion after all the players have been introduced). Cellular expression of GK is governed by a single gene with two promoters (21–23). One of these operates constitutively in

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GK-containing glucose-sensing endocrine cells (homeostatically most important in the pancreatic β-cell but also demonstrated in pancreatic α-cells, enteroendocrine cells, and pituitary gonadotropes) and in neurons of the hypothalamus and the brainstem. The other promoter controls hepatic GK expression but in a process absolutely dependent on the presence of insulin. The kinetic and molecular biological characteristics of GK are the basis of the glucose sensor role of this enzyme in pancreatic β-cells and are a critical determinant of the threshold for glucose-stimulated insulin release (GSIR) close to 5.0 mmol/L (3,24–26). 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 this triad (Fig. 1). 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 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 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. The role of insulin in this induction process is debatable. The physiological chemistry of the glucagon-producing α-cells, which play an essential part in glucose homeostasis, is not well understood (27,28; Fig. 1). α-Cells are

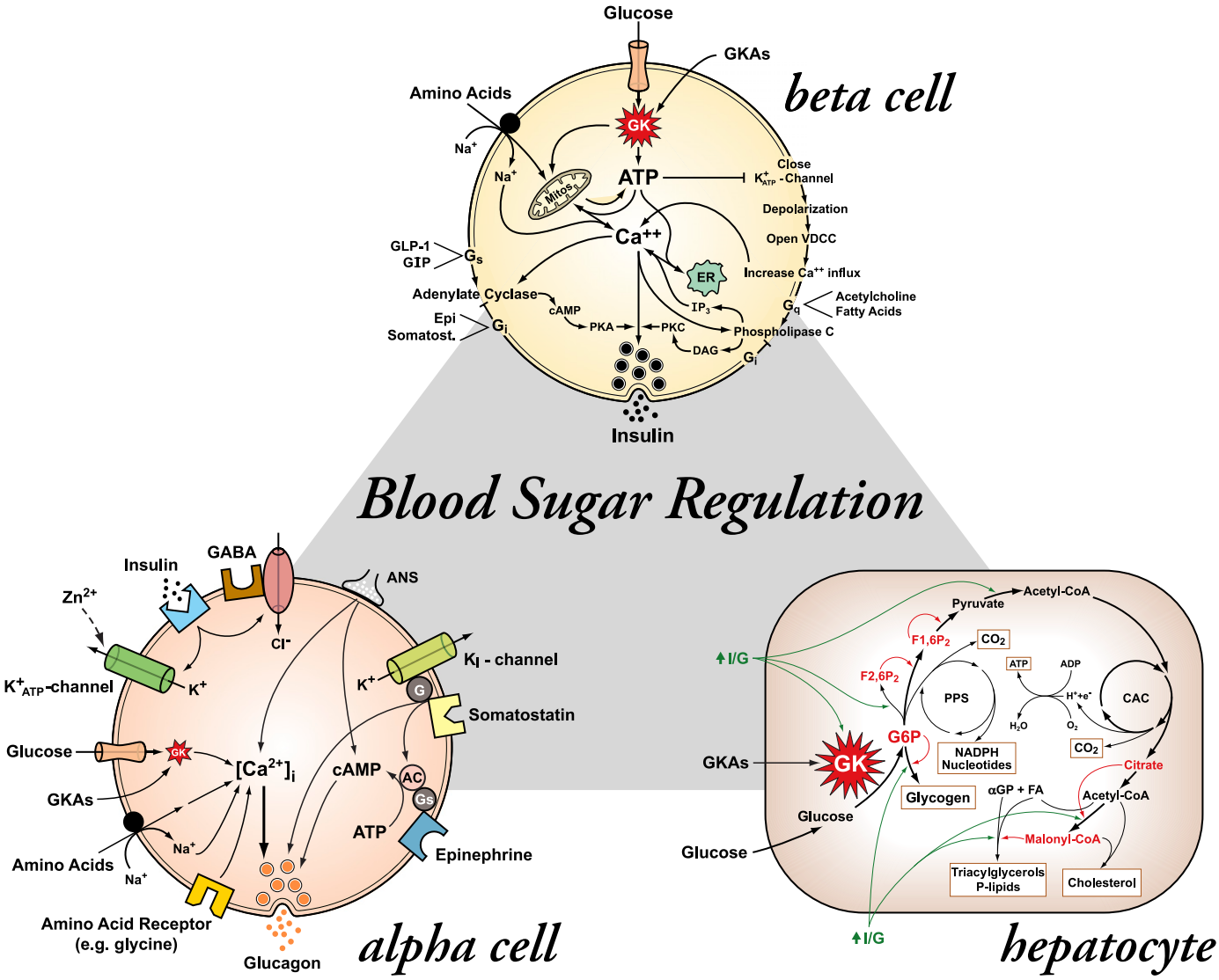

Figure 1—*The GK containing α- and β-cells and hepatocytes are central to glucose homeostasis. The graph shows minimal models of these three cell types (24–30). Note that the α-cell model is highly speculative. Unusual abbreviations used here are defined as follows: αGP, α-glycerol-phosphate; AC, adenylate cyclase; ANS, autonomic nervous system; CAC, citric acid cycle; DAG, diacylglycerol; ER, endoplasmic reticulum; F1,6P$_2$, fructose 1,6-bisphosphate; F2,6P$_2$, fructose-2,6-bisphosphate; FA, fatty acids; G6P, glucose 6-phosphate; G, G_i, G_q, and G_s, various G-proteins; GABA, γ-aminobutyrate; I/G, insulin/glucagon ratio; IP$_3$, inositol 3-phosphate; K_i-channel, G-protein–coupled inward rectifying potassium channel; mitos, mitochondria; PKA, protein kinase A; PKC, protein kinase C; PPS, pentose-phosphate shunt; VDCC, voltage-dependent calcium channel.*

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. It is reemphasized in this context that GK is expressed in these cells, but its function has not been explored sufficiently. In the liver, GK plays a role that is functionally different from that outlined for the β-cell (29,30). In excess of 99% of the body's GK complement resides in the liver, explaining its critical role postprandially in the high-capacity clearance of glucose from the bloodstream coupled with enhanced glycogen synthesis and glucose catabolism (Fig. 1). In hepatocytes, GK is subtly regulated by GKRP, a 68-kDa primarily nuclear protein that inactivates and facilitates the sequestration of GK in the nucleus when glucose levels are low (29–34). 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 enteroendocrine K- and L-cells; and in gonadotropes and thyrotropes.

The central role of GK in glucose homeostasis is perhaps most compellingly demonstrated by the biochemical genetic knowledge that emanated from studying glucokinase disease, which is caused by autosomal dominantly inherited activating and inactivating mutations of the *GK* gene (35). 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. Approximately 600 unique GK mutations have been identified to date (35). All but 1 of the 17 currently known activating GK mutations are located in a circumscribed structural region of GK, conceptualized here as an allosteric modifier region that is clearly separate from the catalytic site (Supplementary Fig. 2). 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 GKRP associates with the enzyme via two amino acid patches in this region with little overlap to the GKA site (15–17,36; B. Zelen. C. Buettger, P. Chen, D. Fenner, J. Bass, C. Stanley, M. Laberge, J.M. Vanderkooi, R. Sarabu, J. Grimsby and F.M.M., unpublished data). Glucose facilitates GKA but inhibits GKRP binding and action. GKA and GKRP binding are apparently mutually exclusive. In liver glucose induces a slow conformational transition that dissociates GKRP/GK but exposes the GKA receptor site (Supplementary Fig. 2). Because islet cells lack GKRP, their influence on GK/GKA interactions should be ignored. The ligand-induced slow transition is illustrated by the effect of GKA on mannoheptulose (MH) binding using tryptophan fluorescence (Fig. 2). MH is a competitive inhibitor of glucose phosphorylation by GK with a dissociation constant of 20 mmol/L, which decreases to 1.26 mmol/L in the presence of near-saturating GKA concentrations. The slow transition is initiated by adding the second ligand (GKA or MH) to a buffered GK solution containing as the first ligand either 1 mmol/L MH or 20 μmol/L GKA, respectively, thus allowing the ternary complex to be formed. The slow component of this process follows first-order kinetics and is temperature-dependent, allowing the calculation of the activation energy for the transition. This step suggests the involvement of one rate-limiting step. 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. We speculate that GKRP would slow down this transition.

GK STATUS IN TYPE 2 DIABETES

—A drug receptor is therapeutically useful only if it is not significantly impaired or deleted by the disease to be treated (3). What is the status of GK in type 2 diabetes? The evidence is strong that β-cell GK is functional in type 2 diabetes, although total GK is probably diminished because β-cell mass and function are reduced as the disease progresses. The known right shift and the blunting of the dose dependency curve of GSIR in type 2 diabetes is probably the result of multiple, yet to be defined defects of the signaling chains controlling GSIR and insulin biosynthesis. The situation of hepatic GK is quite another matter. Hepatic GK expression is totally insulin dependent and may be greatly reduced in severe forms of type 2 diabetes, as demonstrated in human and animal models of the disease (37–39). Still, whatever GK might remain available or is newly induced by GKA-stimulated insulin release (or perhaps by insulin treatment) is probably responsive to the action of GKAs. Nothing is known about the status of GK in other cells expressing the enzyme, but it is speculated that constitutive expression of GK is probably minimally altered by the disease.

MORE ON GKA ACTION AT THE MOLECULAR, CELLULAR, ORGAN, AND WHOLE-BODY LEVELS

—The preceding discussion on the molecular biochemistry of GK and of the GK system in health and disease should leave no doubt that pharmacological activation of this enzyme has a high potential for the treatment of type 2 diabetes. Available results with GKAs support this view (15–17,39–43). GKAs are in general small molecules with considerable chemical structure variety (see three classic examples in Supplementary Fig. 1), but most of them 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, with at least one of them aromatic. The third attachment is a 2-aminoheterocyle or *N*-acyl urea moiety that provides the basis of forming an electron donor/acceptor interaction with R63 of GK. GKAs increase the affinity of recombinant GK for glucose by as much as 10-fold and may also augment the V_{max} moderately, perhaps as much as twofold. The affinity for MgATP is increased when tested at glucose levels below the $S_{0.5}$. Some GKAs lower the Hill coefficient such that they render the enzyme more like hexokinase. The potency of known GKAs varies considerably, with dissociation constants ranging from low nmol/L to low μmol/L. As already discussed, GKAs usually do not bind to GK in the absence of glucose. GKAs potentiate the competitive glucose reversal of GK inhibition by

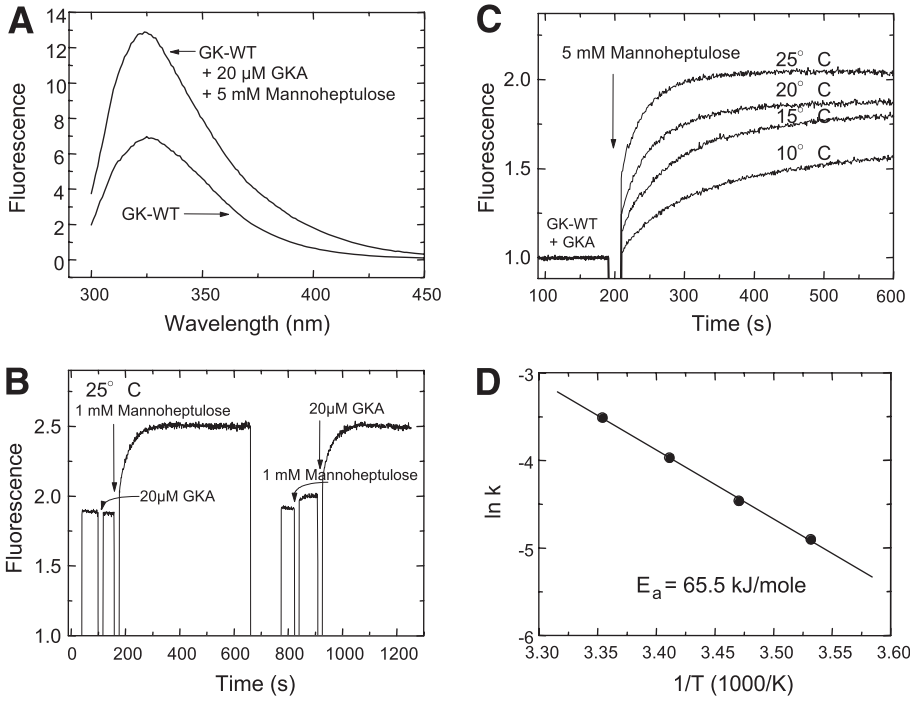

Figure 2—*Tryptophan (W) fluorescence measurements illustrate the process of slow ligand-induced conformational transition of GK.* A*: Tryptophan fluorescence of ~1 μmol/L recombinant human wild-type islet GK in the absence and in the presence of 5 mmol/L MH plus 20 μmol/L cpd A (Supplementary Figure 1). Note that the GKA is at near saturation level, which increases the GK affinity for MH ~15-fold, such that 5 mmol/L MH is near saturation.* B*: It is shown that the slow W-fluorescence increase requires the presence of both the sugar and the activator. Note, however, that 1 mmol/L MH causes a fast but small fluorescence increase, whereas GKA has no effect.* C*: Temperature dependency of the MH/GKA-induced transition process. The tracings are normalized. The transitions have a fast and a slow component. The fluorescence contribution of the fast one diminishes as the temperature falls. The slow one exhibits first-order kinetics, and lowering the temperature decreases the rate constant linearly but does not affect the fluorescence Δ.* D*: Arrhenius plot of the data in* C *allowing the calculation of the activation energy of the slow process. Comparable results were obtained with* D*-glucose. The results are consistent with a model in which the formation of the GK/GKA complex is rate-limiting.*

GKRP, but binding of GKRP and GKAs to GK is probably mutually exclusive (44). Little is known about the GKA effects on the interaction with cellular organelles (e.g., mitochondria or insulin granules [45–48]) or other macromolecules besides GKRP (e.g., the proapoptotic BAD [a member of the Bcl-2 family of proteins] or the bifunctional enzyme that generates and hydrolyzes fructose-2,6-bisphospate [45,46,49]). The proposed molecular interaction with BAD is particularly intriguing in the context of mounting evidence that glucose metabolism and therefore GK activation may be critically involved in β-cell hyperplasia as an adaptive response to chronic elevation of blood glucose. This is an emerging concept that raises questions about the mechanism and significance of glucotoxicity in the molecular pathogenesis of type 2 diabetes (50–52).

The pharmacological effects of GKAs at the cellular level are determined by the physiological role of the enzyme in a particular cell type. In studies with isolated pancreatic islets, GKAs potentiate glucose stimulation of insulin release, the stimulation of respiration by glucose, the oxidation of glucose, and the elevation of free cytosolic calcium caused by glucose (53; Fig. 3). They also enhance the permissive action of glucose for other physiological stimuli including amino acids, fatty acids, acetylcholine, and glucagon-like peptide 1. GKAs potentiate glucose induction of islet GK expression. It can be extrapolated that they increase glucose stimulation of insulin biosynthesis. GKAs improve or even normalize GSIR (Fig. 4) and glucose stimulation of respiration (not shown here) in perifused islets isolated from the pancreas of patients with type 2 diabetes. With isolated islets from normal pancreas donors, 3.0 μmol/L piragliatin shifted the threshold for 3.75 mmol/L GSIR to the left, with no effect on the steepness and the maximum dose-response curve. Note the sharply defined threshold at 5.65 mmol/L glucose in the control without the drug present. In one experiment using islets from a diabetic organ donor, 3.0 μmol/L piragliatin changed the character of the concentration dependency of GSIR. The rather flat rise of the concentration dependency curve was normalized to one showing a steep rise with a well-defined threshold indistinguishable from the control (compare Fig. 4*B* with Fig. 4*D*). These results with control and diabetic islets alike illustrate the high degree of precision in glucose sensing by the β-cell. Studies with isolated rat hepatocytes demonstrated that GKAs stimulate glycolysis and glycogen synthesis and effectively dissociate the nuclear GK/GKRP complex (54).

In intact control animals and a wide variety of animal models of type 2 diabetes, GKAs lowered blood glucose and stimulated insulin release. They also curbed hepatic glucose production in normal and diabetic rats (4). In a long-term study with C57BL/6J mice, GKAs had the remarkable result of preventing the development of diet-induced diabetes with little effect on the weight gain of the animals (55). Results of human trials with healthy subjects or patients with diabetes showed that GKAs reduced blood glucose dose-dependently and increased

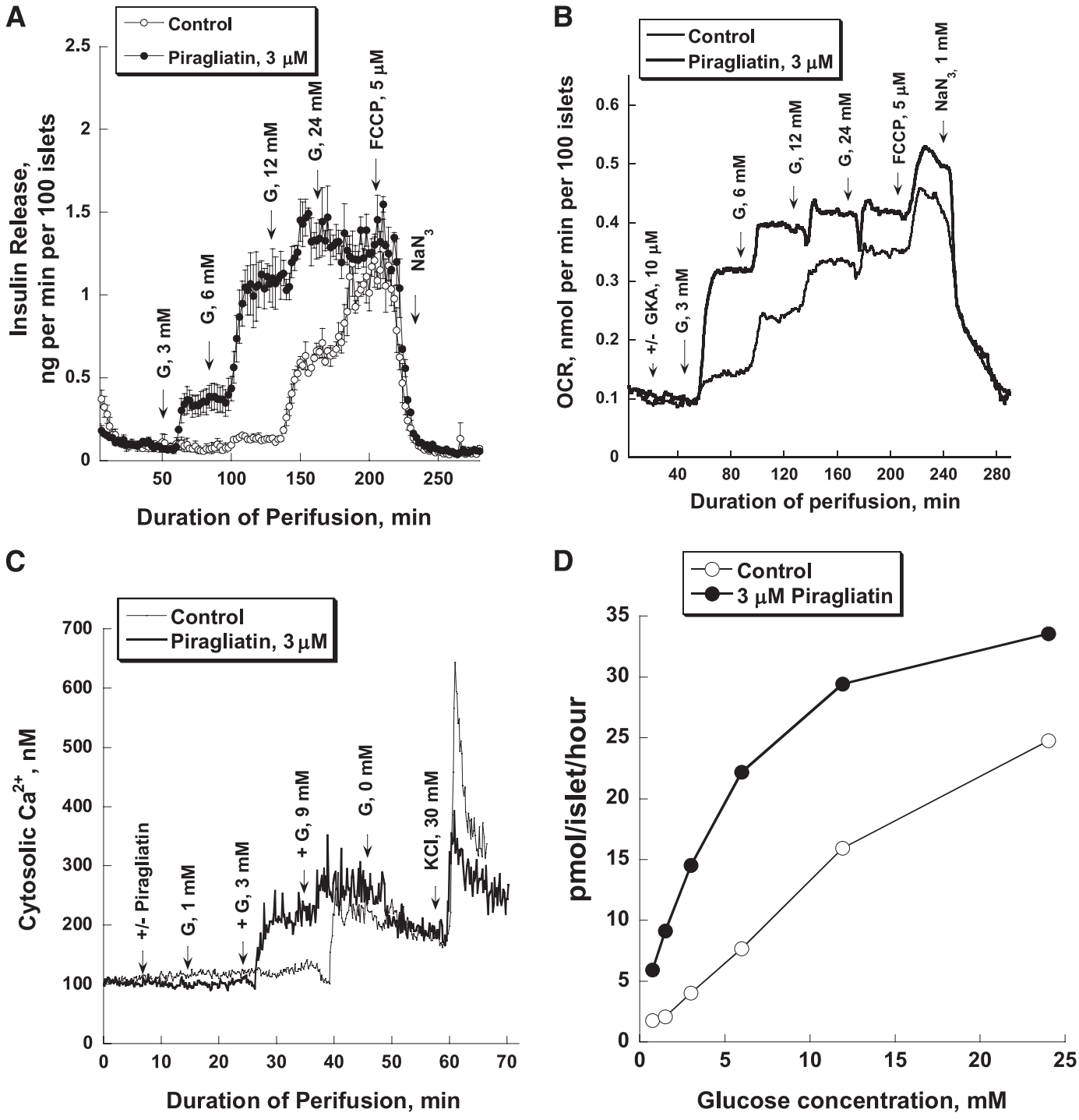

Figure 3—*Effect of piragliatin (cpd M of Supplementary Figure 1) on GSIR, glucose-stimulated respiration, increased glucose oxidation, and elevation of cytosolic calcium in isolated cultured mouse islets.* A *and* B *show data from perifusion studies with a glucose staircase design in which GSIR and* O_2 *were measured in the same experiment. Free cytosolic calcium* (C) *was determined with the Fura-2 method, and glucose oxidation was measured with [U-14C]glucose using standard procedures* (D). *Results in* A, B, *and* D *are the means of three to four experiments, and* C *shows a typical result. OCR, oxygen consumption rate.*

insulin secretion (5,6,56). These trials were performed with two different GKAs: RO-4389620 (cpd M or piragliatin) from Hoffmann La-Roche in a 5.5-day multiple ascending dose study and ARRY 403 from Array Biopharma in a 1-day single ascending dose study. In both trials, blood glucose was lowered dose dependently and normalization of blood glucose was achieved at the highest dose applied. Moderate hypoglycemia was observed at the higher dosages.

PERSPECTIVE—The published results of treating type 2 diabetic patients with GKAs for periods of up to 1 week demonstrate that these agents lower blood glucose effectively in a dose-dependent manner without medically significant side

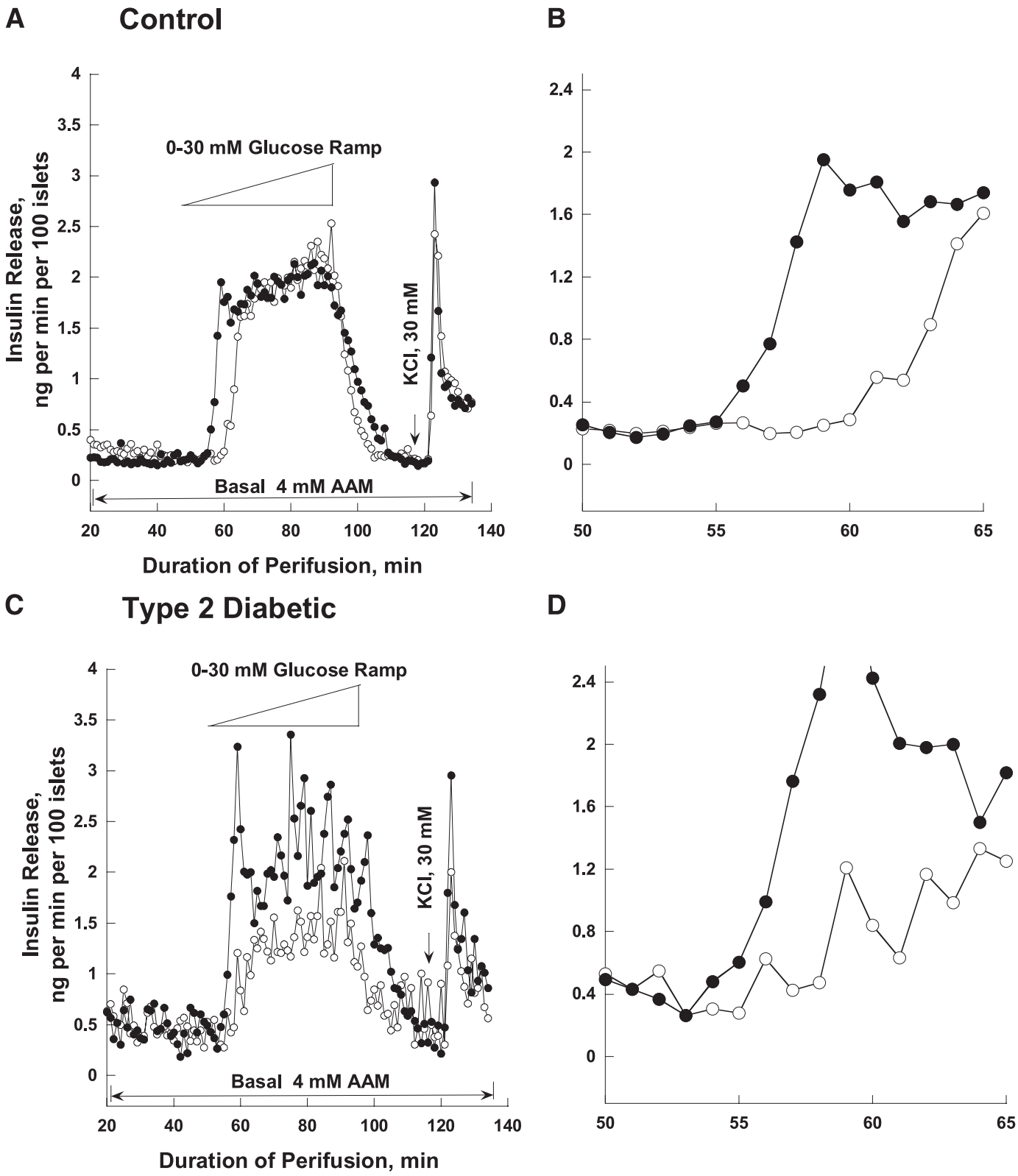

Figure 4—*Effect of piragliatin on GSIR of perifused human pancreatic islets isolated from normal organ donors* (A *and* B, n = *4) and from one case with type 2 diabetes* (C *and* D). *The basic perifusion solution contained a 3.5 mmol/L physiological amino acid mixture plus 0.5 mmol/L glutamine. A glucose ramp with a 0.75 mmol/L/min slope was applied for 40 min, later followed by high potassium. The* right panels *show critical portions of the release profiles for reasons of better clarity. Open symbols are in the absence of and closed symbols are in the presence of piragliatin.*

actions except (not surprisingly) moderate hypoglycemia at the higher doses. Thus, based on a sound basic science foundation, intensive research and development has succeeded in producing a class of powerful antidiabetic drugs with a new mechanism of action contrasting it to all other available pharmacotherapies including insulin. It remains to be seen whether this novel class of drugs withstands the test of time and secures for itself a significant place among approved antidiabetic medicines. Of possible concern is the development of hypoglycemia, fatty liver, and hyperlipidemia. Available results of preclinical and clinical studies suggest, however, that these risks are manageable. Hypoglycemia can probably be greatly reduced by designing GKAs that have a less marked effect on the glucose $S_{0.5}$ than that achieved with current very active experimental GKAs, minimizing alterations in the enzyme cooperativity or by dose titration when such potent agents are used. There is risk of inducing hepatic lipidosis due to enhancement of glycolysis and glucose oxidation, which might result in elevated hepatic malonyl-CoA levels and a switch from fatty acid oxidation to fatty acid and complex lipid biosynthesis, but there is no preclinical evidence for this effect using GKAs. Indications are strong that several GKAs now in development will be advanced to long-term clinical trials that should settle such safety concerns. Finally, looking beyond the primarily medical aspects of GKA-related research, it should be appreciated that the discovery of this new class of agents and the characterization of their action at the molecular, cellular, and organismic level represents in itself a remarkable increase in our understanding of glucose homeostasis with high potential for further advances.

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No potential conflicts of interest relevant to this article were reported.

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