Glibenclamide inhibits islet carnitine palmitoyltransferase 1 activity, leading to PKC-dependent insulin exocytosis

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Hypoglycemic sulfonylureas, such as glibenclamide, have been widely used clinically in the treatment of patients with type 2 diabetes mellitus for the past 40 years, but there is still much controversy about their mode of action (27). In 1984, the existence of glucose-regulated K^+ channels in the β-cell plasma membrane, serving as gated pores controlling transmembrane K^+ fluxes, was demonstrated (36). In the same year, it was found that these ion channels are sensitive to inhibition by ATP and sulfonylureas (36). ATP, or the ATP-to-ADP ratio, thus provides a link between glucose metabolism and changes in β-cell electrical activity elicited by the sugar. More specifically, ATP generated by glucose metabolism may close K^+ channels, resulting in β-cell depolarization and subsequent influx of Ca^{2+} through voltage-activated Ca^{2+} channels located in the plasma membrane, an event that sets in motion secretory granule translocation and the rapid exocytotic discharge of insulin (36).

Experiments have unveiled that glucose retains the ability to release insulin even in the presence of maximally depolarizing concentrations of K^- and diazoxide, an opener of ATP-regulated K^+ (K\text{ATP}) channels (18, 25). Thus signaling molecules other than ATP and Ca^{2+} may be involved in glucose sensing in the β-cell, although the precise nature of such signals has remained elusive. Additionally, sulfonylureas promote insulin exocytosis from permeabilized cells (14), suggesting K\text{ATP}-independent actions also of these drugs. Although poorly defined, this occurs by effects exerted on the secretory machinery itself not involving closure of K\text{ATP} and initiation of Ca^{2+}-dependent electrical activity (14). Moreover, sulfonylureas stimulate insulin exocytosis in β-cells from sulfonylurea receptor-deficient mice, an effect particularly pronounced at high concentrations of glucose (Berggren P-O, unpublished observations).

Furthermore, reports show that >90% of glibenclamide-binding sites are localized intracellularly in the β-cell (7, 31). Interestingly, under chronic treat-
ment, glibenclamide specifically and progressively accumulates in islets in association with secretory granules and mitochondria and causes long-lasting stimulation of insulin secretion (21).

In this study, we set out to determine whether glibenclamide also, in part, acts independently of the KATP-dependent pathway in the β-cell and may directly affect specific intracellular targets, controlling fuel partitioning, to stimulate insulin exocytosis. In doing so, we focused on a pivotal enzyme implicated in fuel partitioning, carnitine palmitoyltransferase 1 (CPT-1; palmitoyl-CoA:l-carnitine O-palmitoyltransferase; EC 2.3.1.21), located in the outer mitochondrial membrane (9). CPT-1 is an important determinant of cellular fatty acid oxidative flux (29, 32). The enzyme catalyzes transfer of long-chain fatty acyl groups from coenzyme A to carnitine and is inhibited by sulfonyleureas in hepatocytes (9, 29, 32). The clinical significance of CPT is illustrated by the fact that CPT deficiency in humans causes fasting hypoglycemia (6), although this may in part be due to hepatic effects. Pioneering studies by McGarry (29) in liver have elucidated the role of CPT-1 in the regulation of hepatic fatty acid oxidation and ketogenesis. His studies also identified malonyl-CoA as an important physiological inhibitor of CPT-1 and an important element in the carbohydrate-induced sparing of fatty acid oxidation (29).

MATERIALS AND METHODS

Materials. Glibenclamide was kindly given by Aventis (Frankfurt, Germany), whereas Schering (Kenilworth, NJ) graciously donated diazoxide. Repaglinide was from Novo-Nordisk (Copenhagen, Denmark). Sodium 2-[b-[4-chlorophenoxyl]-hexyl]oxirane-2-carboxylate (etomoxir, B-82733) was generously donated diazoxide. Repaglinide was from Novo-Nordisk (Copenhagen, Denmark), whereas porcine125I-labeled insulin (28) was raised in guinea pigs in our laboratory. Crystalline rat insulin was from Novo (Copenhagen, Denmark), whereas porcine 125I-labeled insulin was made in our laboratory. [1-14C]palmitate, 1-[methyl-3H]carnitine, and Unisolve were from DuPont-NEN (Boston, MA). Packard Instruments (Downers Grove, IL) provided hyamine hydroxide 10-X. Kieselgel 60 plates and all other chemicals of analytical grade were obtained from Merck (Darmstadt, Germany). Islet cell preparation and culture. Pregnant Wistar rats, purchased from B & K Universal (Sollentuna, Sweden), were killed by cervical dislocation on day 21 of gestation and the fetuses rapidly removed. Islets were prepared from pancreatic glands as previously described (20, 39). Briefly, the pancreata were finely chopped and digested for a short time with collagenase. The carefully washed digest was plated in culture dishes allowing cell attachment (Nunc, Roskilde, Denmark) and cultured for 5 days at 37°C in a humidified atmosphere of 5% CO2 in ambient air in medium RPMI 1640 containing 11.1 mM glucose, 10% fetal calf serum, 2 mM l-glutamine, 100 U/ml benzylpenicillin, and 0.1 mg/ml streptomycin. At the end of the culture period, groups of islets were transferred to fresh medium containing 1% FCS and cultured free-floating overnight, a procedure that minimizes fibroblast proliferation. Spherical islets, free of connective tissue, were then selected under a stereomicroscope and used for the different analyses listed below. In each experiment, all test groups received the same amount of solvent used to dissolve various drugs. We chose to use fetal islets to obtain islet tissue enriched in β-cells; previous studies have shown that fetal islets contain 90–95% β-cells, which display a full secretory response to glucose after a 5-day culture period similar to that of the adult β-cell (20, 39).

Analysis of β-cell CPT-1 mRNA and enzyme activity. Islet cell expression of CPT-1 mRNA was assessed by Northern blot analysis essentially as described (4, 17). Activity of CPT in islets was assayed by measuring the incorporation of tritium-labeled carnitine into acylcarnitine by using a modification of the procedure previously used for liver mitochondria (17). The low protein content of islet preparations in culture necessitated using high specific radioactivity and high concentration of carnitine. Islet samples of 0.2–0.3 mg of protein each were homogenized in 125 μl of lysis buffer. They were then swiftly transferred to Eppendorf tubes containing 0.5 ml of a Krebs-Ringer bicarbonate buffer (26) supplemented with 10 mM HEPES (pH 7.2) by use of a micro dounce homogenizer. Whole homogenates were assayed for CPT activity. Each assay contained, in a total volume of 500 μl, 10 μl of islet protein, 82 mM sucrose, 70 mM KCl, 70 mM imidazole (pH 7.0), 1 mg of BSA, 2 mM l-carnitine (2 μCi/μmol l-[methyl-3H]carnitine), 0.5 μg of antimycin A, 100 μM myristoyl-CoA, 2 mM ATP, and 2 mM MgCl2. Reactions were carried out at 37°C for 20 min. Assays were linear with respect to time up to 35 min and were also linear with respect to protein in the range assayed.

Analysis of β-cell fatty acid oxidation. Duplicate groups of 25 islets, labeled overnight with [1-14C]palmitate (10 μCi/ml) to achieve sufficient uptake and steady state, were incubated in small glass vials at 37°C for 2 h in 100 μl of a Krebs-Ringer bicarbonate buffer (26) supplemented with 10 mM HEPES (pH 7.2), 3 or 20 mM d-glucose, glibenclamide, or etomoxir. For measurements of fatty acid oxidation, reactions were terminated by the addition of 100 μl of 0.05 mM antimycin A in ethanol. The 14CO2 formed was released from incubation medium by the addition of 100 μl of 0.4 M Na2HPO4 (pH 6.0) and trapped in 250 μl of hyamine. After overnight postincubation, the radioactivity was measured by liquid scintillation counting.

Lipid extraction and quantification of diacylglycerol. Groups of 250–300 islets were cultured free-floating overnight in RPMI 1640 medium supplemented with 1% fetal calf serum. Islets were preincubated for 45 min at 37°C in KRBB buffer. They were then swiftly transferred to Eppendorf tubes containing 1 ml KRBB buffer (pawarmed to 37°C) supplemented with 40 μM glibenclamide. Islets were then incubated for the indicated time period, rapidly pelleted, and quickly rinsed once in ice-cold PBS. Tubes were then immediately plunged into liquid nitrogen and kept frozen at −80°C pending further analysis of their diacylglycerol (DAG) content. After samples on ice were thawed, islets were sonicated in a 500-μl extraction solution consisting of chloroform-methanol-HCl (100:100:1, vol/vol/vol) and 100 μl of PBS with 10 mM EDTA. After centrifugation (5 min, 12,000 g), the aqueous phase was removed and reextracted with 100 μl of chloroform, which were added to the organic phase. The combined chloroform phases were evaporated under a stream of liquid nitrogen and resolubilized in 50 μl of the chloroform solution. This solution was reextracted with 10 μl of PBS with 10 mM EDTA and then reevaporated. Samples were then stored at −80°C under nitrogen until analyzed for DAG. 1,2-DAGs were quantified as described (39). Briefly, dried lipids were solubilized in 20 μl of an octyl-β-D-glucoside-
cardiolipin solution (7.5% octyl-β-D-glucoside, 5 mM cardiolipin in 1 mM diethylenetriaminepentaacetic acid) by sonication in a bath sonicator. The reaction was then carried out in 100 µl containing 20 µl of sample solution, 50 mM imidazole HCl (pH 6.6), 50 mM NaCl, 12.5 mM MgCl₂, 1 mM EDTA, 2 mM DTT, 6.6 µg DAG kinase, and 1 mM [γ-32P]ATP for 30 min at room temperature. Lipids were extracted and evaporated as above. Samples were then run on Kieselgel 60 plates activated by preheating at 120°C. Plates were developed with chloroform-methanol-acetic acid (65:15:5, vol/vol/vol) and subjected to autoradiography. Standard samples of [1-1,2-dipalmitoyl] were run in parallel. The intensities of the spots corresponding to phosphatidic acid were quantified using densitometry and are expressed as arbitrary units (optical density).

PKC translocation assay. Groups of 100 cultured islets were incubated for 30 min in RPMI 1640 medium plus 10% FCS with the various additions indicated in Fig. 5. The islets were quickly washed with cold PBS and used directly for preparation of membrane and cytosol fractions as described by Alcázar et al. (1). Membrane and cytosol proteins were separated on a 9% SDS-PAGE gel and transferred to a nitrocellulose membrane. The membranes were incubated with mouse anti-PKC monoclonal antibody (MC5, Santa Cruz Biotechnology, Santa Cruz, CA) followed by incubation with a horseradish peroxidase-linked secondary antibody. Antibody binding was visualized using the enhanced chemiluminescence immunoblotting detection system (Amersham International). Band intensities were quantified by densitometry, and the results are expressed as percentage of the membrane-to-cytosol ratio of control islets.

Insulin exocytosis determinations. Duplicate groups of 10 islets were preincubated for 45 min in KRHB buffer (26) containing 3 mM glucose at 37°C. They were then incubated for another 30 min in fresh medium, supplemented as indicated in Fig. 6. To circumvent any influence of KATP channels on membrane potential and cytosolic Ca²⁺ concentration, cells were incubated under Ca²⁺-clamped conditions, i.e., in the presence of 20 mM glucose, 25 mM KCl, and 400 µM diazoxide (18). The insulin concentration in incubation medium was analyzed radioimmunologically (22). Dextran-coated charcoal in 0.2 M glycine buffer was used to separate bound and free insulin. Interassay coefficient of variation (CV) was 2.8% at 21 µU/ml insulin and 2.3% at 104 µU/ml (n = 28, duplicates). Intra-assay CV was 1.2% at 20 µU/ml insulin and 0.8% at 125 µU/ml (n = 16, duplicates). Standard curve range was 3.9–250 µU/ml insulin.

Statistical analysis. Results presented are derived from independent experiments performed on different days. Means ± SE were calculated and groups of data compared using Student’s t-test.

RESULTS

Expression of CPT-1 mRNA species in rat islets. Figure 1 shows that rat islets and clonal rat insulinoma INS-1 cells express exclusively the liver isozyme of CPT-1 mRNA (CPT-1α), with an approximate size of 4.7 kb, whereas no expression of the muscle-type CPT-1 transcript (CPT-1β) is detected. Glibenclamide dose-dependently inhibits CPT enzymatic activity. As shown in Fig. 2A, glibenclamide and malonyl-CoA dose-dependently inhibit CPT enzymatic activity in islet homogenates, the apparent IC₅₀ occurring at ~50 µM for glibenclamide and at 25 µM for malonyl-CoA. It should be noted that the enzyme assays were performed in the presence of albumin (2 mg/ml), which tightly binds glibenclamide. This was done because CPT enzyme activity measurements require the addition of protein because of the minute amount of tissue available. Hence, the plots grossly underestimate the inhibitory potency of glibenclamide (~10-fold on the basis of assays of recombinant CPT in the presence and absence of BSA; Fig. 2B). Another important implication of this study is that glibenclamide inhibits both CPT-1 and CPT-2 in islets and that each enzyme is identically sensitive because it binds at the active site, not the malonyl-CoA site. Therefore, when 5 µM free glibenclamide inhibits CPT-1 by 50% and CPT-2 by 50%, the total effect on the pathway is inhibition of >50%, because two enzymes in the same pathway are being inhibited. This can be seen from the plot (Fig. 2C) in which extrapolation of inhibition to infinite concentration leads to 100% inhibition of CPT activity (CPT-1 and CPT-2 inhibited) by glibenclamide, whereas extrapolation of inhibition by malonyl-CoA to infinitely high concentration leads to only ~50% inhibition (only CPT-1 inhibited). Another KATP channel-blocking insulin secretagogue, repaglinide, was also tested and was found not to affect CPT enzymatic activity at 10 or 50 µM (data not shown). Repaglinide possesses many of the physicochemical properties of glibenclamide but does not affect KATP-independent insulin secretion.

Glibenclamide inhibits islet fatty acid oxidation. As shown in Fig. 3, exposure to glibenclamide (at 3 mM glucose) for 90 min causes a substantial suppression of the oxidation of endogenous fatty acids in islets prelabeled with [14C]palmitate, amounting to an ~40% reduction of control values. Such a reduction of mitochondrial β-oxidation is expected from the finding of CPT inactivation by glibenclamide. This inhibitory effect is comparable in magnitude to that caused by high glucose. Additionally, the specific CPT-1 inhibitor etomoxir (in 3 mM glucose), serving as a positive control, causes an ~70% reduction in the rate of palmitate oxidation.
Rapid DAG formation by glibenclamide exposure. Reducing CPT-1 activity, and thereby fatty acid oxidation, with glibenclamide should lead to a shunting of acyl-CoA esters to lipid esterification products. That this indeed occurs in the sulfonylurea-exposed islets is amply illustrated in Fig. 4. Thus glibenclamide stimulation of intact islets results in a robust and transient accumulation of DAG. The effect is significant already at 10 min, being maximally sixfold enhanced at 30 min and leveling off by 60 min of glibenclamide exposure.
Glibenclamide translocates PKC to membranes. To assess whether accelerated DAG formation by glibenclamide is associated with a biological action, we measured PKC activation (as estimated by its translocation from cytosol to membranes) because DAG activates the classical isoforms of this enzyme. Entirely consistent with the findings above, stimulating islets with glibenclamide causes a dose-dependent activation of PKC, shown as translocation of the enzyme from the cytosol to membranes (Fig. 5, A and B). At 5 μM, glibenclamide enhances PKC activity approximately twofold, a figure that is further increased to threefold at 40 μM of the drug (Fig. 5, A and B). In comparison, 10 nM of the phorbol ester TPA elicits a 2.6-fold translocation of PKC. The PKC antibody used detects the classical enzyme isoforms expressed in islets, i.e., α, β, and γ.

Glibenclamide-stimulated insulin exocytosis occurs partly through K<sub>ATP</sub>-independent and PKC-dependent pathways. Figure 6 reports short-term experiments in which insulin exocytosis is measured in Ca<sup>2+</sup>-clamped conditions (i.e., presence of elevated glucose and K<sup>+</sup>) with the K<sub>ATP</sub> channels bypassed (presence of diazoxide). Even under these Ca<sup>2+</sup>-clamped conditions, glibenclamide causes a robust 50% stimulation of insulin secretion at 20 mM glucose. A similar 150% increase is noted in response to the PKC-activating phorbol ester TPA (10 nM). Additionally, no additive effects between glibenclamide and TPA occur, arguing indirectly in favor of a common mechanism of action of these two agents. Etomoxir (50 μM), a specific CPT-1 inhibitor, stimulates insulin secretion to the same extent as glibenclamide (Fig. 6). Again, no additive effects between etomoxir- and glibenclamide-stimulated insulin release are detected. Finally, the coaddition of the PKC inhibitor H-7 (10 μM) completely prevents the insulinotropic actions of both TPA and glibenclamide (Fig. 6). Similar findings were obtained under Ca<sup>2+</sup>-clamped conditions at 3 mM glucose. Thus, in a separate series of experiments in 3 mM glucose, etomoxir (50 μM) stimulates insulin secretion (46 ± 7% over basal; n = 6, P < 0.05) to the same extent as glibenclamide (48 ± 6% over basal; n = 6, P < 0.05). Again, no additive effects between etomoxir- and glibenclamide-stimulated insulin release are detected (not shown). In their entirety, these findings indicate that glibenclamide can promote insulin secretion through K<sub>ATP</sub> channel-independent mechanisms and that this stimulation is in part dependent on the activation of PKC elicited by the sulfonylurea after fat oxidation inhibition.

In an attempt to assess the quantitative importance of this novel PKC-dependent and K<sub>ATP</sub>-independent pathway in glibenclamide-stimulated insulin exocytosis at different glucose concentrations, we compared the extent to which glibenclamide promotes exocytosis under normal vs. Ca<sup>2+</sup>-clamped conditions at low and high glucose in a separate series of nine observations. Thus, at 3 mM glucose, glibenclamide (40 μM) stimulates insulin exocytosis 602 ± 116%, a figure that was reduced to 120 ± 39% in the additional presence of 25 mM KCl and 400 μM diazoxide. At 20 mM glucose, glibenclamide stimulated insulin exocytosis 46 ± 7%, a figure that was reduced to 21 ± 3% in the additional presence of 25 mM KCl and 400 μM diazoxide. Hence, the results indicate that the K<sub>ATP</sub> channel-independent pathway accounts for −20% (120 vs. 602%) of the maximal insulin-releasing capacity of glibenclamide at 3 mM glucose; an effect that increases to −45% (21 vs. 46%) in 20 mM glucose, figures that are in fair agreement with capacitance measurements in mouse islets in a previous report (14).

**DISCUSSION**

A pivotal enzyme implicated in fuel partitioning is CPT-1, located in the outer mitochondrial membrane...
CPT-1 activity is an important determinant of cellular fatty acid oxidative flux (29, 32). The enzyme catalyzes transfer of long-chain fatty acyl groups from coenzyme A to carnitine. A specific carnitine-acylcarnitine carrier translocates long-chain acylcarnitines into the mitochondrial matrix, where they are reesterified to acylthioesters by CPT-2. Acylthioesters undergo \( \beta \)-oxidation, generating reducing equivalents used to

\[ \text{Reducing equivalents} \]

\[ \rightarrow \text{Reactions in mitochondria} \]

\[ \text{Reducing equivalents} + \text{Oxygen} \rightarrow \text{Water} + \text{Energy} \]

...
produce ATP via oxidative phosphorylation (29, 32). The enzymatic activity of CPT-1 is inhibited by malonyl-CoA, generated during glucose metabolism (10, 29, 32, 33). This inactivation switches the routing of endogenous fatty acids from mitochondrial β-oxidation to the biosynthesis of complex lipids such as DAG and phosphatidic acid (10, 32, 33). Glucose-stimulated de novo synthesis of DAG after malonyl-CoA-induced CPT-1 inhibition may thus couple glucose metabolism to insulin exocytosis (10, 28, 32, 33, 38). Here, we have shown that glibenclamide, globally the most widely prescribed sulfonylurea, exerts an inhibitory action similar to that of malonyl-CoA on islet CPT-1 enzymatic activity. However, in contrast to malonyl-CoA, glibenclamide seemingly also inhibits CPT-2 activity in the islets. Our previous study (9) indicated that the primary effect of glibenclamide was probably on CPT-1, since the drugs are less potent in inhibition of CPT in fasting and diabetic animals. Also, when testing inhibition of CPT-2, we used detergents to break the membranes and expose CPT-2, at the same time killing the CPT-1 activity by the detergent action. It is unclear whether glibenclamide gets into the mitochondria.

We also noted a suppressed rate of fatty acid oxidation by both glucose and glibenclamide, as expected from their inhibitory effect on CPT activity. When fatty acid oxidation is decreased, the acyl-CoA esters are diverted to the biosynthesis of esterified lipid products. Entirely consistent with this scenario is our observation of a rapid and marked accumulation of DAG in response to glibenclamide and the resultant PKC activation. With respect to PKC action in the β-cell, its activation by glucose-derived DAG or phorbol esters may promote insulin exocytosis by controlling the phosphorylation of several key proteins, e.g., voltage-dependent Ca²⁺ channels (2, 3). Previous reports indicate that islet DAG mass is not affected by variations in Ca²⁺ (12, 30, 40). Likewise, islet inositol 1,4,5-trisphosphate levels are not altered by artificially stimulating Ca²⁺ influx by ionomycin or K⁺ or by blocking it with EGTA (5, 42). Furthermore, islet PKC activity is only minimally affected by changing Ca²⁺ from sub-nanomolar to submicromolar concentrations (34). All these findings make it unlikely that the observed increase in DAG levels and PKC activity by glibenclamide would be secondary to Ca²⁺ influx stimulated by the sulfonylurea. Additionally, any Ca²⁺ influence on DAG levels and PKC activity obviously has no functional significance for insulin release, since glibenclamide (and TPA) evoked a robust increase in insulin secretion even under Ca²⁺-clamped conditions. Also, the secretory response to glibenclamide under Ca²⁺-clamped conditions was nonadditive with TPA and blocked by the PKC inhibitor H-7, thus clearly indicating that the KATP-independent effect of glibenclamide on insulin exocytosis is PKC mediated and not affected by Ca²⁺.

The clinical significance of CPT is illustrated by the fact that CPT deficiency in humans causes fasting hypoglycemia (6). Additional evidence supporting the view that CPT inhibition is linked to β-cell stimulation by glucose can be derived from the fact that, when insulin secretion is suppressed, such as during chronic hyperlipidemia, the CPT-1 gene is upregulated in association with enhanced fat oxidation in the β-cell (4). The importance of CPT-1 in β-cell glucose signaling is further underscored by the finding that blocking the penultimate step (ATP-citrate lyase) in malonyl-CoA synthesis curtails glucose-sensitive insulin release (8, 15). Conversely, CPT-1 overexpression in INS-1 insulinoma cells not only results in exaggerated fatty acid oxidation rates but also impaired glucose-stimulated insulin secretion (37).

Our findings are compatible with the view that glibenclamide, through its intracellular inactivation of CPT-1, in part promotes insulin exocytosis via KATP-independent and PKC-dependent pathways. Also consistent with this view is the observation that the specific CPT-1 inhibitor etomoxir caused similar effects to glibenclamide on insulin secretion and fat oxidation and that their effects are not additive. This new KATP-independent effect of glibenclamide is quantitatively significant and glucose dependent. Differences in lipophilicity and uptake rates may affect the timing by which the different agents activate PKC. Thus, if TPA (the more lipophilic drug) activated PKC faster than glibenclamide (as one would expect), it would trigger insulin exocytosis more rapidly and give rise to a larger amount of insulin being released over 30 min, as was observed in this case. Glibenclamide seems to be exceptional among the sulfonylureas in that it specifically and progressively accumulates in islets and associates with secretory granules and mitochondria, causing long-lasting stimulation of insulin secretion (21). It is conceivable that the inactivation of CPT-1 may explain the KATP-independent and PKC-dependent insulin secretion by sulfonylurea reported previously (14, 35). This possibility is also in accord with previous reports indicating a hypoglycemic effect of fatty acid oxidation inhibitors in vivo (16). It was recently shown that acyl-CoA activates atypical forms of PKC (43), which may be consistent with our present findings of PKC involvement in glibenclamide-stimulated secretion. Nonetheless, a direct stimulatory effect of long-chain acyl-CoA esters on the exocytotic process (11) may also be involved in the effects of glibenclamide noted herein. Additionally, a direct effect of sulfonylurea on PKC activity was ruled out in a previous study (41).

In hepatocytes, the sensitivity of CPT-1 to malonyl-CoA inhibition is increased by insulin and decreased in diabetes (9, 17). Whether a reduced sensitivity of CPT-1 to malonyl-CoA inhibition in the β-cell, resulting in decreased DAG production and deficient PKC activation, may contribute to the impaired glucose-stimulated insulin secretion characterizing human type 2 diabetes mellitus is an obvious possibility that remains to be tested.

The physiological significance of our results remains to be determined. At first glance, it would seem that the submicromolar therapeutical concentrations of
glibenclamide maximally achieved in the postabsorptive state (23, 24) would not be sufficient to affect the islet CPT system in diabetic patients (Fig. 2). Interestingly, however, under chronic treatment, glibenclamide progressively and selectively accumulates in islets and is slowly cleared from islets on drug withdrawal (21). Additionally, there are large interindividual variations in the pharmacodynamics and pharmacokinetics of glibenclamide, which are furthermore greatly influenced by genetic polymorphisms of the cytochrome P-450 2C9 system (24). Hence, carriers of certain CYP2C9 genotype variants show markedly reduced clearance rates of glibenclamide, resulting in severalfold-elevated serum concentrations of the drug (24). There are also several important drug interactions that may additionally elevate glibenclamide serum levels, which may be significant in elderly diabetic patients who are often on multiple medications and have impaired drug metabolism (23, 27). Add to this the high levels normally achieved of biologically active and long-acting glibenclamide metabolites that may also impact the CPT system (23). Thus it cannot be excluded that the inhibitory effect of glibenclamide on islet CPT activity described here may contribute to the sustained hypoglycemic effect of the drug observed in some diabetic patients, even after drug removal for 24–48 h, to which no explanation has been given to date. Repaglinide, a KATP channel-blocking insulin secretagogue possessing many of the physicochemical properties of glibenclamide but without affecting KATP-independent insulin secretion, did not affect CPT activity. We went on to test all the sulfonylureas that we could obtain and found that, for each drug, the potency of inhibition of CPT corresponded to that drug’s potency as an effective anti-diabetic agent (not shown). In Ref. 9, we showed inhibition by glibenclamide and tolbutamide with the most potent acting sulfonylurea on insulin secretion, glibenclamide, being the more potent CPT inhibitor. The inactive metabolite of tolbutamide carboxytolbutamide is not a CPT inhibitor (9).

Furthermore, the specific CPT-1 inhibitor etomoxir stimulates insulin secretion nonadditively with glibenclamide both in our hands (Fig. 6) and in previous reports from other groups (8, 44). Moreover, etomoxir elevates DAG contents (19), adding further credence to our concept. Glibenclamide also inhibits CPT-1 enzymatic activity in the liver (9). Whether liver output of lipids is stimulated by glibenclamide in vivo is difficult to address conclusively, because such an effect may be counteracted systemically by the portal delivery of insulin elicited by glibenclamide-induced insulin release from the pancreas occurring when the drug is administered in vivo.

In conclusion, we suggest a model in which islet β-cell CPT-1 activity is reduced by glibenclamide, thereby diverting fatty acid metabolism from mitochondrial oxidation to the biosynthesis of DAG, which causes KATP-independent and PKC-dependent exocytosis of insulin. We suggest that chronic CPT inhibition, through the progressive islet accumulation of glibenclamide, may explain the prolonged stimulation of insulin secretion in some diabetic patients, even after drug removal, that contributes to the sustained hypoglycemia of the sulfonylurea. Whether this mechanism also results in lipid overload in the β-cell, causing β-cell lipopapoptosis (13, 32) explaining the clinical phenomenon of “secondary failure” to sulfonylureas (27), is currently being evaluated in our laboratory.

DISCLOSURES

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