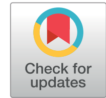

METABOLISM

Central K_{ATP} Channels Modulate Glucose Effectiveness in Humans and Rodents

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Hyperglycemia is a potent regulator of endogenous glucose production (EGP). Loss of this "glucose effectiveness" is a major contributor to elevated plasma glucose concentrations in type 2 diabetes (T2D). K_{ATP} channels in the central nervous system have been shown to regulate EGP in humans and rodents. We examined the contribution of central K_{ATP} channels to glucose effectiveness. Under fixed hormonal conditions (studies using a pancreatic clamp), hyperglycemia suppressed EGP by ~50% in both humans without diabetes and normal Sprague-Dawley rats. By contrast, antagonism of K_{ATP} channels with glyburide significantly reduced the EGP-lowering effect of hyperglycemia in both humans and rats. Furthermore, the effects of glyburide on EGP and gluconeogenic enzymes were abolished in rats by intracerebroventricular administration of the K_{ATP} channel agonist diazoxide. These findings indicate that about half of the suppression of EGP by hyperglycemia is mediated by central K_{ATP} channels. These central mechanisms may offer a novel therapeutic target for improving glycemic control in subjects with T2D.

Although type 2 diabetes (T2D) affects over 400 million people worldwide, many fundamental questions remain about abnormal glucose metabolism, and approximately half of patients are suboptimally treated (1). Endogenous glucose production (EGP) is essential for maintaining plasma glucose concentrations in the fasted state and is the main source (providing up to 80%) of elevated glucose in T2D (2). "Glucose effectiveness" is the ability of glucose per se to suppress EGP, independent of its effects on insulin secretion (3). Its relevance to glucose metabolism has been well established by many research groups (3–10). In humans who do not have diabetes, hyperglycemia inhibits EGP to nearly the same degree irrespective of insulin level (4). In the presence of identical and constant plasma insulin, glucagon, and growth hormone concentrations, a doubling of the plasma glucose level (to ~10 mmol/L) inhibited EGP by nearly half in subjects who did not have diabetes (5,6). Of particular note, we and others have demonstrated that glucose effectiveness is impaired in subjects with T2D (5,7,11–13). Paradoxically, EGP is elevated despite sustained hyperglycemia; loss of glucose effectiveness may thus account for ~70 g of excess glucose produced overnight in these individuals (5).

Growing evidence indicates that glucose exerts its effects on EGP both through direct inputs to the liver and via higher signals emanating from the central nervous system (CNS). Within the liver, regulation of EGP by hyperglycemia probably occurs at the level of the glucose-6-phosphate pool, which is regulated by relative flux through glucokinase and glucose-6-phosphatase (5). Additionally, the hypothalamus possesses specific types of glucose-sensing neurons whose activity is either increased or decreased by glucose (14). A balance in the activity of these glucose-sensing neurons is likely critical for regulating EGP (10). Various nutrients and hormones, including glucose and insulin, have been shown to regulate EGP in rodents by modulating hypothalamic K_{ATP} channels (15–17). In glucose-excitatory hypothalamic glucose-sensing neurons, increased glucose metabolism raises the

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ATP-to-ADP ratio and closes the K_{ATP} channel. Glucose has been shown to open K_{ATP} channels in certain hypothalamic glucose-inhibited neurons, potentially by increasing intracellular pyruvate concentrations (18), in association with reductions in EGP (16). This all supports a critical role for central mechanisms in modulating glucose effectiveness.

The relevance of these findings to humans was first highlighted by parallel studies of nondiabetic rats and humans without diabetes, in whom activation of extrapancreatic K_{ATP} channels reduced EGP under euglycemic conditions (19). Diazoxide was used to activate K_{ATP} channels under a "pancreatic clamp" in which pancreatic hormone secretion was inhibited by somatostatin (19). Under these fixed hormonal conditions, diazoxide significantly and progressively suppressed EGP in both humans and rats (19). In rats, inhibition of EGP by oral diazoxide was reversed by intracerebroventricular (ICV) administration of a K_{ATP} channel antagonist, suggesting that such an effect is centrally mediated (19). Subsequent human studies demonstrated a similar time course and extent of suppression of EGP by intranasal insulin, further supporting the role of the CNS in regulating glucose and energy metabolism in humans (20–23). However, regulation of EGP by central K_{ATP} channels seems to be impaired in humans with T2D and in diabetic rats (24). This suggests that a loss of this regulation contributes to hyperglycemia in diabetes and that central pathways could be an important target for new diabetes treatments (25).

The purpose of this study was to determine to what extent hyperglycemia suppresses EGP via central K_{ATP} channels. The K_{ATP} channel antagonist glyburide blunted the suppression of EGP through hyperglycemia in both humans and rats. Concomitant ICV administration of the K_{ATP} channel activator diazoxide restored glucose effectiveness, confirming that it is, at least in part, centrally mediated.

RESEARCH DESIGN AND METHODS

All procedures used for working with the humans and rodents in this study were approved by the Albert Einstein College of Medicine Institutional Review Board and the Einstein Institutional Animal Care and Use Committee, respectively.

Human Studies

Study Design

Nine healthy adults who did not have diabetes, had no family or personal medical history of diabetes, and were not currently taking any medications were enrolled in the study (four men, five women; age, 37.4 ± 15.0 years; BMI

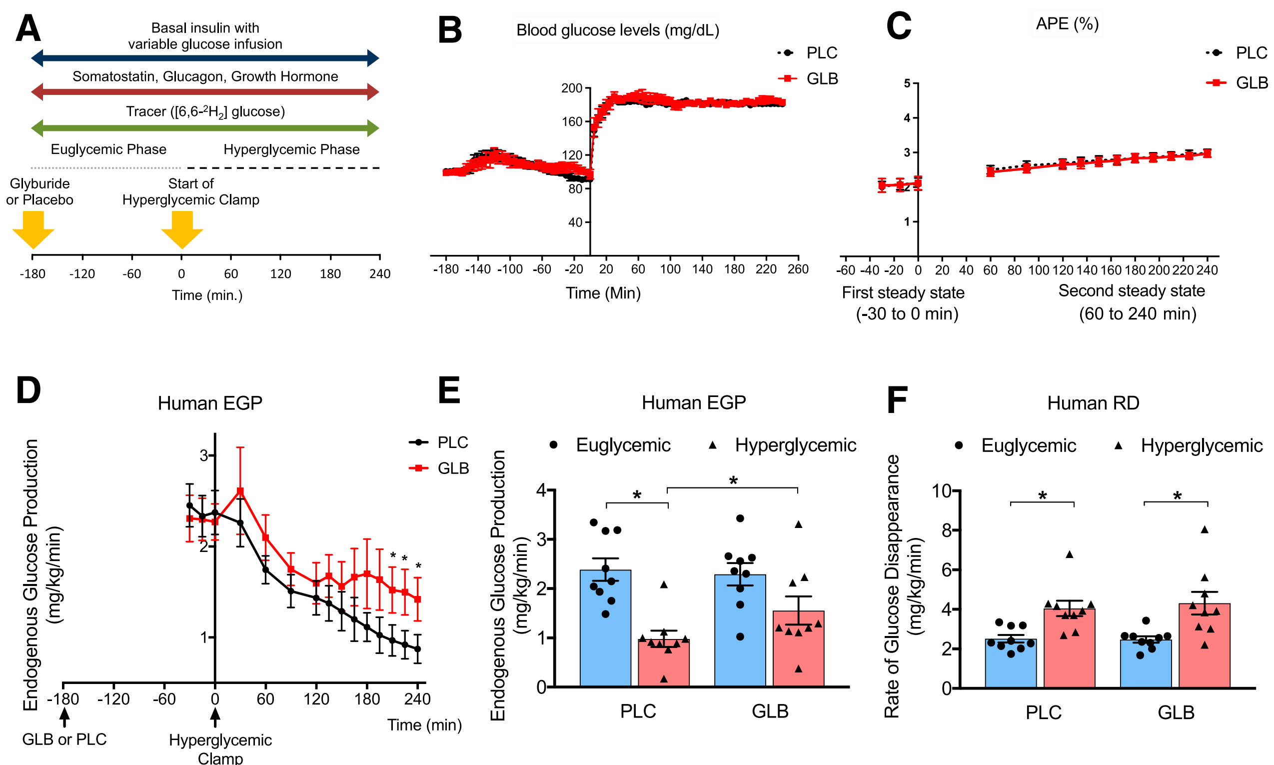

Figure 1—Effect of K_{ATP} channel inhibition on the ability of glucose effectiveness to regulate EGP and glucose disposal in humans. *A*: Schematic of the hyperglycemic pancreatic clamp protocol used in humans (n = 9). *B*: Blood glucose levels during the clamps. *C*: Atom percent enrichment (APE) during the steady-state period. *D*: Time course of EGP at the euglycemic baseline (t = −30 to 0 min) and during the hyperglycemic clamp (t = 0–240 min). *E*: Mean EGP during the final hour of the hyperglycemic clamp studies ("Hyperglycemic") and at the euglycemic baseline ("Euglycemic"). *F*: R_d during the final hour of the hyperglycemic clamp. *$P < 0.05$, repeated-measures ANOVA (*D*) or two-way Student t test (*E* and *F*). Data are the mean ± SEM. GLB, glyburide; PLC, placebo.

mean ± SEM 25.9 ± 4.5 kg/m^2). Each participant underwent a pair of identical 7-h euglycemic-hyperglycemic clamp studies; the studies was performed at least 5 weeks apart in random order. After administration of either oral glyburide (10 mg) or identical placebo capsules at $t = -180$ min, a pancreatic clamp in which basal glucoregulatory hormones were replaced was initiated with infusion of somatostatin (72 ng/kg/min), insulin (1 mU/kg/min), glucagon (0.6 ng/kg/min), and growth hormone (3 ng/kg/min).

Pancreatic Clamp Studies

Subjects underwent two paired hyperglycemic pancreatic clamp studies separated by at least 5 weeks. All subjects arrived at the clinical research center after an overnight fast, and one indwelling venous catheter was inserted in each arm: an 18-gauge catheter was inserted in an antecubital vein for infusions, and a contralateral hand vein was cannulated for obtaining blood samples (26). To obtain arterialized venous blood samples, that contralateral hand was maintained at 55°C in a thermoregulated sleeve.

On the basis of pilot studies in which optimal metabolic effects of glyburide were observed ~6 h after oral administration of the drug, our experimental protocols lasted 7 h and consisted of an initial 3-h euglycemic pancreatic clamp followed by a 4-h hyperglycemic pancreatic clamp (Fig. 1*A*). At the beginning of the study ($t = -180$ min), subjects received either oral glyburide (10 mg) or matched placebo capsules (with an appearance identical to that of the glyburide) in a randomized, single-blinded fashion. At the same time, infusions of basal insulin and somatostatin, with replacement of glucoregulatory hormones (glucagon [0.6 ng/kg/min] and growth hormone [3 ng/kg/min]) and 20% dextrose (infused variably if needed to maintain euglycemia) were initiated. Informed by our previous studies in which glucagon levels rose slightly above fasting levels during clamp conditions (19,26), glucagon infusion rates were reduced from 1 ng/kg/min to 0.6 ng/kg/min in the current studies. In so doing our subjects successfully maintained fasting glucagon levels throughout the clamp studies.

The dose of somatostatin was selected in order to optimally suppress C-peptide levels in the presence of both hyperglycemia and glyburide. After carefully reviewing the literature, we selected a somatostatin infusion rate of 72 ng/kg/min (27)—a 44% increase from that used in our prior studies. This rate successfully prevented significant increases in endogenous insulin levels, although there were small rises in C-peptide with combined glyburide and hyperglycemia (see RESULTS). Of the 9 subjects who completed the paired clamp studies, one was excluded because C-peptide levels were not suppressed during hyperglycemia.

In order to measure glucose fluxes, [6,6-^{2}H2]glucose was administered via a primed infusion (200 mg/m^2/min for 3 min, then 4 mg/m^2/min thereafter) throughout the entirety of the clamp studies. Glucose kinetics and the estimated volume of distribution of glucose were used in order to calculate the bolus and continuous infusion rates for [6,6-^{2}H2]glucose by using the mathematical models developed by Finegood et al. (28) and Steele (29).

Once basal insulin infusion rates were established and "steady-state" euglycemic conditions were achieved, glucose metabolism was quantified during the euglycemic phase of the clamp. After 3 h of euglycemia, the hyperglycemic portion of the clamp was initiated (at $t = 0$ min) and was continued for 4 h. Plasma glucose levels were rapidly increased to ~180 mg/dL via a variable infusion of labeled 20% dextrose. Once steady-state hyperglycemic conditions were achieved, glucose kinetics were quantified during the hyperglycemic phase of the clamp.

Throughout the entire 420 min of the study, plasma glucose concentrations were measured at 5- to 10-min intervals. From $t = -180$ to $t = -30$ min, optimal insulin infusion rates were established by making frequent adjustments (approximately every 25 min) in order to maintain euglycemia, with the intent of preventing the need for exogenous glucose infusion. In three subjects in whom glucose levels fell below the target (90 mg/dL) during the final hour of euglycemia, consistent with our previous studies we infused small amounts of glucose to avoid precipitous drops in glucose that could stimulate counterregulatory hormone release and thereby impact EGP (24). Most of the mean glucose infusion rate was accounted for by the rate for one subject who required glucose infusion in both studies to avoid drops in glucose; excluding this subject did not impact the EGP results.

Despite the occasional need for small amounts of exogenous glucose infusion, basal insulin levels were still achieved. With an anticipated portal insulin–to–systemic insulin ratio of ~2.4:1 (30), and a mean fasting systemic insulin level of ~11 μU/mL in our subjects, we would expect a corresponding mean fasting portal insulin level of ~25 μU/mL. Such a portal insulin level would have been attained with insulin infusion during our clamp studies, as the systemic insulin levels in our studies were ~19–23 μU/mL. Because insulin infusion through a peripheral vein in depancreatized dogs resulted in portal insulin levels that approximated (within 20%) systemic insulin levels (31), it is likely that the liver was exposed to insulin levels similar to subjects' habitual fasting levels.

An individual's optimal insulin infusion rate was kept constant between $t = -30$ and 0 min. At $t = 0$ min, we initiated a bolus of 20% dextrose, which was designed to increase the plasma glucose concentration to 180 mg/dL within 7.5 min. Specifically, a volume-based bolus (0.7 multiplied by the subject's weight) was administered, half over the first 2.5 min and the subsequent half over the next 5 min. Optimal 20% dextrose infusion rates intended to maintain plasma glucose levels at ~180 mg/dL were established in a similar fashion between $t = 0$ and 180 min, with the goal of keeping 20% dextrose infusion rates constant between $t = 180$ and 240 min. From $t = -180$ to 240 min, blood samples were obtained for determining plasma glucose, insulin, glucagon, C-peptide,

cortisol, growth hormone, free fatty acids (FFAs), glycerol, lactate, and [6,6-^{2}H2]glucose.

All infusions except 20% dextrose were stopped at t = 240 min. Meals were provided to each participant after the clamp, and glucose was strictly monitored after the study was completed; 20% dextrose infusion rates were decreased as appropriate in order to maintain euglycemia. Subjects who received glyburide were admitted overnight to the clinical research center for hourly blood glucose monitoring, and they continued to receive 20% dextrose infusions as needed to maintain euglycemia.

Glucose Turnover

Glucose turnover data present the mean values during the steady-state portion of the study, as previously described. R_a, R_d, and other indices of glucose turnover were estimated by using the Steele equation (6). Assuming that $R_a = R_d$ in a steady state, we used the following calculation: R_d = (Rate of basal [6,6-^{2}H2]glucose infusion + [20% dextrose/Rate of {6,6-^{2}H2}glucose infusion])/Atom percent enrichment fraction/weight (kilograms). EGP was determined by subtracting the rate of glucose infusion from the tracer-derived R_a. Gas chromatography–electron impact mass spectrometry were performed at the Clinical and Translational Science Awards (CTSA) Analytic Core Laboratory in order to determine [6,6-^{2}H2]glucose (32). Plasma samples for gas chromatography–mass spectrometry were derivatized after protein precipitation to the aldehyde pentaacetate with hydroxylamine hydrochloride–acetic anhydride.

Plasma Hormone and Substrate Determinations

Plasma glucose, insulin, C-peptide, FFA, and glucagon levels were measured as previously described (19). Radioimmunoassay was used in order to measure plasma cortisol levels (Immuno-Biological Laboratories America, Minneapolis, MN) (33).

Rodent Studies

Study Design

Nineteen male Sprague-Dawley rats (age, 12–14 weeks; weight ± SEM, 386.1 ± 7.2 g; Charles River Laboratories)

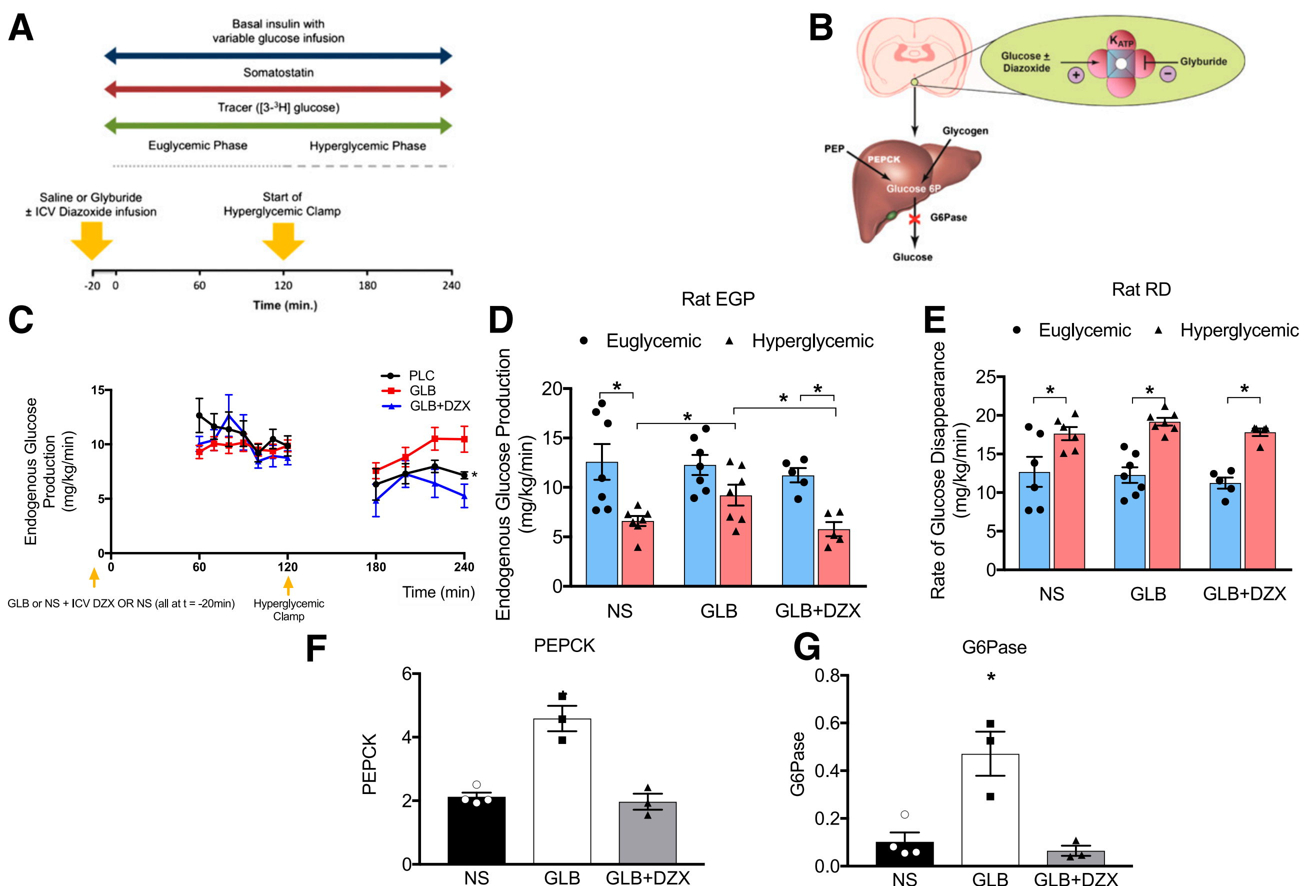

Figure 2—Effect of K_{ATP} channel inhibition on the ability of glucose effectiveness to regulate EGP and glucose disposal in rodents. *A*: Schematic of the hyperglycemic pancreatic clamp protocol used in rats (n = 19). *B*: Schematic of the opposing actions of glyburide and diazoxide at various sites within the same K_{ATP} channel. *C*: Time course of EGP at the euglycemic baseline (t = 60–120 min) and during the hyperglycemic clamp (t = 180–240 min). *D*: Mean EGP during the hyperglycemic clamp studies and at the euglycemic baseline. *E*: R_d during the final hour of the hyperglycemic clamp. *F* and *G*: Expression of *Pepck* (*F*) and *G6Pase* (*G*) in the rodents receiving normal saline (NS) (n = 7), glyburide (GLB) (n = 7), or glyburide and ICV diazoxide (GLB+DZX) (n = 5). *P < 0.05, ANOVA with Bonferroni correction. Data are the mean ± SEM. Glucose 6P, glucose-6-phosphate; PEP, phosphoenolpyruvate.

were used in this study. Cannulae were inserted for ICV, then clamp studies were performed. One week after cannulae placement, animals underwent a second surgery to place arterial and venous catheters (19). After they recovered, the animals were divided into three experimental groups: group 1 (n = 7) received saline (oral gavage), group 2 (n = 7) received oral glyburide, and group 3 (n = 5) received glyburide (oral gavage) and an ICV infusion of the K_{ATP} channel agonist diazoxide.

Pancreatic Clamp Studies

All rats underwent a 4-h hyperglycemic pancreatic clamp. The rats were anesthetized and received either saline or glyburide (2.5 mg/kg) ~20 min before the clamp (Fig. 2*A*). At $t = -20$ min, ICV infusion of diazoxide (0.08 μL/min; group 3) or comparable volumes of artificial cerebral spinal fluid (groups 1 and 2) were initiated. A primed continuous intravenous infusion of [3-^{3}H]glucose was initiated at t = 0 and maintained for 4 h (240 min) in order to assess glucose kinetics. While maintaining peripheral basal insulin levels, a hyperglycemic clamp was performed during the final 2 h (t = 120–240 min) (33). Before the infusion study ($t = -20$ min), rats were anesthetized with isoflurane and received normal saline or glyburide (2.5 mg/kg) through an oral gavage. For the remainder of the studies, the rats were conscious and unrestrained.

An ICV infusion of diazoxide (9.69 μg/μL at 0.006 μL/min) or saline was started at $t = -20$ min. At t = 0 min (~20 h after saline or glyburide gavage), a primed continuous intravenous infusion (400 μL/min for 3 min, 40 μL/min thereafter) of [3-^{3}H]glucose (Perkin Elmer) was begun and maintained throughout the study in order to assess glucose kinetics in vivo. To assess the specific activity of [3-^{3}H]glucose, plasma was sampled during the time period when a steady state was anticipated. A euglycemic clamp, with a target glucose level of 95–120 mg/dL, was performed during t = 0–120 min, and a hyperglycemic clamp (target glucose level, 250–270 mg/dL) was performed during t = 120–240 min. During the final 2 h of the infusion study (t = 120–240 min), a peripheral basal insulin (1 mU/kg/min) pancreatic hyperglycemic clamp was performed, as previously described (12). At the onset of the hyperglycemic clamp (t = 120 min), plasma insulin levels were comparable among groups: group 1 (saline), 0.75 ± 0.10 ng/mL; group 2 (glyburide), 0.91 ± 0.08 ng/mL; group 3 (glyburide and diazoxide), 0.72 ± 0.08 ng/mL ($P >$ 0.05 for all groups). The data for insulin, C-peptide, and glucose are presented as the mean of values obtained at multiple time points during the infusions. After the study, rats were anesthetized with ketamine (150 mg/kg). Liver tissue samples were obtained by freeze clamping.

Real-time RT-PCR

In order to examine the expression of *Pepck* and *G6pase* in rat liver, real-time RT-PCR was performed by using a Roche LightCycler and SYBR Green I (Qiagen), as previously described (19). Expression was also examined for the "housekeeping genes" *B2m*, *Rpl-19*, *Gapdh*, *β-actin*, and *18s*. Five housekeeping genes were used in order to normalize the gene expression data. The primer sequences are provided in Supplementary Table 1. Relative gene expression was calculated as the ratio of expression of the target gene divided by the geometric mean of the expression of housekeeping genes (19,34).

Statistical Analysis

We performed a paired, two-way Student t test to compare glyburide and placebo studies in the human subjects and repeated-measures ANOVA to compare multiple time points within the same study type. Rodent studies were analyzed by using one-way ANOVA to compare the main effects. Post hoc pairwise comparisons were performed as appropriate using Bonferroni tests. P values <0.05 were considered significant. Data are presented as the mean ± SEM.

Data and Resource Availability

All data generated or analyzed during this study are included in this article and the Supplementary Material.

RESULTS

Extrapancreatic K_{ATP} Channels Play a Substantial Role in the Regulation of EGP in Humans

Given the importance of glucose effectiveness in regulating EGP, we examined whether central K_{ATP} channels contribute to the suppressive effects of hyperglycemia. Paired studies were conducted in which subjects who did not have diabetes (n = 9) received either glyburide (10 mg) or placebo on separate occasions. A "pancreatic clamp" was used in order to attain basal levels of insulin and glucoregulatory hormones; this was particularly important in preventing the stimulatory effects of glyburide and hyperglycemia on insulin secretion (Fig. 1*A*). EGP was measured during a 3-h period of euglycemia (glucose, 90 mg/dL), and again during a subsequent 4-h period of hyperglycemia (glucose, 180 mg/dL). Blood glucose levels and the percentage enrichment of plasma glucose with deuterated [6,6-$^{2}H_2$]glucose (atom percent enrichment) were stable throughout both euglycemia and hyperglycemia, confirming steady-state conditions (Fig. 1*B* and *C*).

Hyperglycemia suppressed EGP by 59% by the final hour of the placebo studies (EGP, 0.98 ± 0.17 mg/kg/min); with baseline euglycemia EGP was 2.38 ± 0.23 mg/kg/min ($P < 0.001$) (Fig. 1*D* and *E*). This is consistent with many previous observations that hyperglycemia potently inhibits EGP in the face of basal insulin levels (3,5,8,9,12,35), and it highlights the importance of glucose effectiveness.

Blocking K_{ATP} channels with glyburide blunted the ability of hyperglycemia to suppress EGP; under these conditions, hyperglycemia suppressed EGP by only 32% (EGP with glyburide, 1.56 ± 0.29 mg/kg/min; EGP with placebo, 0.98 ± 0.17 mg/kg/min; P = 0.047) (Fig. 1*D* and *E*). Although substantial glucose infusion rates were required to maintain hyperglycemia, they were lower with

glyburide, which is consistent with the higher EGP in those studies (Table 1). Similar insulin, FFA, cortisol, and glucagon concentrations were maintained throughout all clamp studies, and somatostatin effectively suppressed C-peptide levels (Table 1).

Extrapancreatic K_{ATP} Channels Do Not Impact Glucose Uptake in Humans

As expected on the basis of previous findings (3,5), there was a rapid doubling of R_d with hyperglycemia. R_d did not, however, differ between glyburide and placebo (R_d during the final hour of the clamp with glyburide, 4.31 ± 0.57 mg/kg/min; with placebo, 4.05 ± 0.39 mg/kg/min; $P = 0.26$) (Fig. 1*F*). These results are consistent with our observations that central activation of K_{ATP} channels does not affect peripheral R_d (19).

The Effects of Glyburide on EGP Are Blocked by ICV Diazoxide in Rats

Complementary rodent studies were performed in order to confirm that the effects of glyburide on EGP are central in origin. We first determined the response of EGP to oral glyburide, then we infused ICV diazoxide to antagonize the effects of glyburide on central K_{ATP} channels. Normal male Sprague-Dawley rats ($n = 19$) were assigned to one of three groups: *1*) oral glyburide, *2*) oral saline, or *3*) oral glyburide with concurrent ICV diazoxide, after establishing the optimal dose and time course of administration for each drug. Similar to the human studies, EGP was comparable among groups during the euglycemic phase of the rodent studies ($P = 0.25$, ANOVA) and was suppressed by about 48% under hyperglycemic conditions (6.60 ± 0.47 mg/kg/min); under euglycemic conditions, EGP was 12.58 ± 1.69 mg/kg/min ($P = 0.031$). Likewise, plasma glucose, insulin, and C-peptide concentrations during the clamps did not differ among groups; insulin infusion rates were also comparable (Table 2). The addition of glyburide blunted the suppressive effects of hyperglycemia on EGP, reducing it from 48% to 25% (EGP during hyperglycemia with glyburide, 9.21 ± 0.75 mg/kg/min; with saline, 6.60 + 0.47 mg/kg/min; $P = 0.046$) (Fig. 2*D*). Thus, inhibiting K_{ATP} channels diminished the suppressive effect of hyperglycemia

Table 1—Plasma hormone concentrations, insulin infusion rate, plasma glucose concentration, and glucose infusion rate in humans

Group	Time (min) 0	120–180	180–240
Insulin (μU/mL)			
Placebo	22.76 ± 2.44	22.82 ± 2.38	22.51 ± 2.74
Glyburide	23.06 ± 4.59	19.13 ± 2.51	20.03 ± 3.11
C-peptide (ng/mL)			
Placebo	0.20 ± 0.02	0.26 ± 0.04	0.26 ± 0.05
Glyburide	0.25 ± 0.04	0.30 ± 0.06	0.44 ± 0.09
FFA (mmol/L)			
Placebo	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01
Glyburide	0.12 ± 0.06	0.10 ± 0.05	0.08 ± 0.01
Cortisol (μg/dL)			
Placebo	13.70 ± 2.54	14.65 ± 2.69	13.70 ± 2.01
Glyburide	13.39 ± 2.00	11.71 ± 1.25	10.75 ± 1.78
Glucagon (pg/mL)			
Placebo	68.04 ± 11.85	65.22 ± 7.37	64.48 ± 7.98
Glyburide	60.82 ± 5.95	58.90 ± 5.29	65.31 ± 4.42
Lactate (mg/dL)			
Placebo	8.16 ± 1.69	7.89 ± 1.41	7.30 ± 1.04
Glyburide	7.74 ± 0.81	7.56 ± 0.84	7.32 ± 0.94
Insulin infusion rate (mU/kg/min)			
Placebo	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Glyburide	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Glucose (mg/dL)			
Placebo	91.3 ± 2.71	182 ± 0.50	181 ± 0.55
Glyburide	97.3 ± 5.72	182 ± 0.55	183 ± 1.32
GIR (mg/kg/min)			
Placebo	0.20 ± 0.17	2.91 ± 0.15	3.03 ± 0.50
Glyburide	0.56 ± 0.23	2.62 ± 0.13*	2.76 ± 0.42*

Data are the mean ± SEM. Repeated-measures ANOVA was used to compare hormone and substrate concentrations between time intervals. A paired *t* test was used to compare glucose and glucose infusion rate (GIR) values among study conditions (separately for euglycemia and hyperglycemia). *Significantly different between placebo and glyburide for that time period ($P < 0.05$, Student *t* test).

on EGP by about half. Simultaneous activation of central K_{ATP} channels with ICV diazoxide abolished the effects of glyburide on EGP (EGP with diazoxide and glyburide, 5.78 ± 0.57 mg/kg/min; EGP with saline, 6.60 ± 0.47 mg/kg/min; $P = 0.12$). EGP during hyperglycemia was significantly different between group 2, receiving glyburide (9.21 ± 0.75 mg/kg/min), and group 3, receiving glyburide and diazoxide (5.78 ± 0.57 mg/kg/min) ($P = 0.034$) (Fig. 2*D*).

Expression of Key Hepatic Gluconeogenic Enzymes in Rats Is Modulated by Central K_{ATP} Channels

Following the hyperglycemic clamp, hepatic *Pepck* expression was significantly higher after glyburide administration (relative gene expression with saline, 2.13 ± 0.13; with glyburide, 4.59 ± 0.40; $P = 0.001$) (Fig. 2*F*), consistent with the effect of glyburide on EGP. This effect of glyburide was reversed by concomitant ICV administration of diazoxide (relative *Pepck* expression with diazoxide and glyburide, 1.97 ± 0.25; $P = 0.88$ vs. saline). Similarly, there was a significant increase in the relative expression of the glucose-6-phosphatase gene (*G6pase*) with glyburide (0.47 ± 0.09); *G6pase* expression with saline was 0.10 ± 0.04 ($P = 0.004$). This increase was also abolished by ICV diazoxide (relative gene expression with diazoxide and glyburide, 0.06 ± 0.02; $P = 0.85$ vs. saline) (Fig. 2*G*). This suggests that central K_{ATP} channel activation increases hepatic phosphorylated STAT3, a known mechanism for regulating gluconeogenic enzyme transcription and translation (19,36,37).

DISCUSSION

These parallel studies in humans and rodents are, to our knowledge, the first to demonstrate that central K_{ATP} channels contribute to glucose effectiveness, a critical component of the regulation of EGP. Consistent with extensive reports in the literature (3,5,8,9,12,35), hyperglycemia suppressed EGP by approximatively half in both humans who did not have diabetes and normal Sprague-Dawley rats under fixed and carefully maintained hormonal conditions. We have shown that either a fourfold increase in the insulin level or a doubling of the plasma glucose level results in a similar ~50% suppression of EGP (7). Collectively, these observations support the importance of glucose per se in the regulation of EGP. Indeed, the relevance of "glucose effectiveness" to the regulation of glucose metabolism has been well established by many research groups (3–10).

Here we show that antagonism of K_{ATP} channels with glyburide significantly reduced the EGP-lowering effect of hyperglycemia in both humans and rats. The effects of glyburide on EGP and gluconeogenic enzymes in rats were abolished by ICV administration of the K_{ATP} channel agonist diazoxide, suggesting that about half of EGP suppression by hyperglycemia is mediated by central K_{ATP} channels. These findings are consistent with previous observations that nutrient and hormonal signals regulate EGP by modulating central K_{ATP} channels (16,19,38,39). This regulation seems to be impaired in T2D (24) and in insulin-resistant humans (40), which is potentially consistent with the observation that expression of the Kir 6.2 subunit is reduced in the medial basal hypothalamus of obese diabetic rats (41). Therefore, future studies examining the role of K_{ATP} channels in modulating glucose effectiveness in human and rodent models of insulin resistance and T2D are clearly warranted.

Indeed, CNS regulation of metabolism is likely to be highly complex, comprising many types of highly specialized neurons and interconnected central and peripheral regulatory pathways. As noted above, glucose has been shown to open K_{ATP} channels in hypothalamic glucose-inhibited neurons (42). The resulting decrease in the activity of these neurons would be expected to reduce EGP, consistent with our current observations; this suggests that glucose-inhibited neurons contribute to glucose effectiveness. Of note, activation of steroidogenic factor-1 neurons (which define the ventromedial hypothalamus) under basal conditions increased whole-body glucose utilization and glucose uptake into skeletal muscle, while simultaneously increasing glucose production, thereby maintaining glucose levels. In contrast, activation of the same steroidogenic factor-1 neurons suppressed gluconeogenic gene expression and glycogen phosphorylase a activity in the liver under hyperinsulinemic conditions. Thus, there are clearly cells in the ventromedial hypothalamus that are associated with both glucose-lowering and glucose-raising mechanisms, which are differentially activated under different metabolic conditions.

Notably, diazoxide inhibits binding of glyburide to the sulfonylurea receptor (43), such that the two substances may compete for binding to the K_{ATP} channel–sulfonylurea receptor (SUR1) complex in the brain. This provides reassurance that even if diazoxide were more immediately available in the CNS, it would still block the binding of glyburide. Therapeutic doses of glyburide, though rapidly extruded from the CNS, reach concentrations in brain

Table 2—Plasma hormone and glucose concentrations in rodents

Group	Time (min) 0–120	180–240
Insulin (μU/mL)		
Normal saline	0.75 ± 0.10	1.47 ± 0.30
GLB	0.91 ± 0.08	1.46 ± 0.16
GLB + DZX	0.72 ± 0.08	1.53 ± 0.18
C-peptide (ng/mL)		
Normal saline	0.14 ± 0.03	0.57 ± 0.11
GLB	0.16 ± 0.02	0.53 ± 0.05
GLB + DZX	0.17 ± 0.02	0.71 ± 0.13
Glucose (mg/dL)		
Normal saline	118.8 ± 8.60	263.57 ± 3.81
GLB	100.35 ± 5.91	273.16 ± 7.43
GLB + DZX	95.60 ± 5.58	253.61 ± 2.40

Data are the mean ± SEM. One-way ANOVA was used to compare values for the three study conditions. DZX, diazoxide; GLB, glyburide.

parenchyma that are substantially higher than the half-maximal effective concentration of SUR1; abundant amounts of glyburide have been detected in cerebrospinal fluid 4 h after intraperitoneal delivery of glyburide (44), which is a time course comparable to that used in this study. Furthermore, biological effects of sulfonylureas often last much longer than their plasma half-life, given the duration of receptor interaction and the formation of active metabolites (45). This is all consistent with our findings that EGP did not change over time in the group that received glyburide and diazoxide. Finally, ICV diazoxide blocked the effects of glyburide on EGP, providing further evidence for regulation of EGP by central K_{ATP}-dependent mechanisms.

The effect of glyburide on EGP became significant after about 200 min of hyperglycemia in the human clamp studies. This delay likely reflects the time required for orally administered agents to interact centrally and for the subsequent effects of central signals to affect the transcription and translation of hepatic gluconeogenic enzymes. This time course is consistent with previous findings demonstrating that central regulation of EGP requires several hours to manifest in humans, in contrast with the more acute changes in EGP that are mediated by insulin and glucose signaling within the liver (19,20). Indeed, the importance of subacute regulation of glucose metabolism is highlighted by several transgenic models, in which chronic disruption of central signals caused abnormal glucose homeostasis (39,46–48). This is evidenced further by the association of various neurologic disorders with abnormal glucose metabolism (24,49–52).

In conclusion, these novel studies in humans and rodents highlight the key role of central signals in the regulation of glucose effectiveness. Given the urgent need for additional treatment options for patients with T2D, central pathways may offer promising therapeutic targets.

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References

1. American Diabetes Association. 8. Pharmacologic approaches to glycemic treatment: *Standards of Medical Care in Diabetes—2018*. Diabetes Care 2018; 41(Suppl. 1):S73–S85
2. Riddle M, Umpierrez G, DiGenio A, Zhou R, Rosenstock J. Contributions of basal and postprandial hyperglycemia over a wide range of A1C levels before and after treatment intensification in type 2 diabetes. Diabetes Care 2011;34:2508–2514
3. Ader M, Ni TC, Bergman RN. Glucose effectiveness assessed under dynamic and steady state conditions. Comparability of uptake versus production components. J Clin Invest 1997;99:1187–1199
4. Wolfe MM, Reel GM. Inhibition of gastrin release by gastric inhibitory peptide mediated by somatostatin. Am J Physiol 1986;250:G331–G335
5. Hawkins M, Gabriely I, Wozniak R, Reddy K, Rossetti L, Shamoon H. Glycemic control determines hepatic and peripheral glucose effectiveness in type 2 diabetic subjects. Diabetes 2002;51:2179–2189
6. Tonelli J, Kishore P, Lee DE, Hawkins M. The regulation of glucose effectiveness: how glucose modulates its own production. Curr Opin Clin Nutr Metab Care 2005;8:450–456
7. Mevorach M, Giacca A, Aharon Y, Hawkins M, Shamoon H, Rossetti L. Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. J Clin Invest 1998;102:744–753
8. Alzaid AA, Dinneen SF, Turk DJ, Caumo A, Cobelli C, Rizza RA. Assessment of insulin action and glucose effectiveness in diabetic and nondiabetic humans. J Clin Invest 1994;94:2341–2348
9. Basu A, Dalla Man C, Basu R, Toffolo G, Cobelli C, Rizza RA. Effects of type 2 diabetes on insulin secretion, insulin action, glucose effectiveness, and postprandial glucose metabolism. Diabetes Care 2009;32:866–872
10. Ferrannini E, DeFronzo RA, Sherwin RS. Transient hepatic response to glucagon in man: role of insulin and hyperglycemia. Am J Physiol 1982;242: E73–E81
11. Basu R, Basu A, Nielsen M, Shah P, Rizza RA. Effect of overnight restoration of euglycemia on glucose effectiveness in type 2 diabetes mellitus. J Clin Endocrinol Metab 1999;84:2314–2319
12. Morton GJ, Matsen ME, Bracy DP, et al. FGF19 action in the brain induces insulin-independent glucose lowering. J Clin Invest 2013;123:4799–4808
13. Nagasaka S, Tokuyama K, Kusaka I, et al. Endogenous glucose production and glucose effectiveness in type 2 diabetic subjects derived from stable-labeled minimal model approach. Diabetes 1999;48:1054–1060
14. Routh VH, Hao L, Santiago AM, Sheng Z, Zhou C. Hypothalamic glucose sensing: making ends meet. Front Syst Neurosci 2014;8:236
15. Carey M, Kehlenbrink S, Hawkins M. Evidence for central regulation of glucose metabolism. J Biol Chem 2013;288:34981–34988
16. Lam TK, Gutierrez-Juarez R, Pocai A, Rossetti L. Regulation of blood glucose by hypothalamic pyruvate metabolism. Science 2005;309:943–947
17. Pocai A, Lam TK, Gutierrez-Juarez R, et al. Hypothalamic K(ATP) channels control hepatic glucose production. Nature 2005;434:1026–1031
18. Gonzàlez JA, Reimann F, Burdakov D. Dissociation between sensing and metabolism of glucose in sugar sensing neurones. J Physiol 2009;587:41–48
19. Kishore P, Boucai L, Zhang K, et al. Activation of K(ATP) channels suppresses glucose production in humans. J Clin Invest 2011;121:4916–4920
20. Dash S, Xiao C, Morgantini C, Koulajian K, Lewis GF. Intranasal insulin suppresses endogenous glucose production in humans compared with placebo in the presence of similar venous insulin concentrations. Diabetes 2015;64: 766–774
21. Benedict C, Brede S, Schiöth HB, et al. Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes 2011;60:114–118
22. Heni M, Wagner R, Kullmann S, et al. Hypothalamic and striatal insulin action suppresses endogenous glucose production and may stimulate glucose uptake during hyperinsulinemia in lean but not in overweight men. Diabetes 2017;66: 1797–1806

23. Gancheva S, Koliaki C, Bierwagen A, et al. Effects of intranasal insulin on hepatic fat accumulation and energy metabolism in humans. Diabetes 2015;64:1966–1975
24. Esterson YB, Carey M, Boucai L, et al. Central regulation of glucose production may be impaired in type 2 diabetes. Diabetes 2016;65:2569–2579
25. Bentsen MA, Mirzadeh Z, Schwartz MW. Revisiting how the brain senses glucose-and why. Cell Metab 2019;29:11–17
26. Hawkins M, Tonelli J, Kishore P, et al. Contribution of elevated free fatty acid levels to the lack of glucose effectiveness in type 2 diabetes. Diabetes 2003;52:2748–2758
27. Shah P, Vella A, Basu A, et al. Elevated free fatty acids impair glucose metabolism in women: decreased stimulation of muscle glucose uptake and suppression of splanchnic glucose production during combined hyperinsulinemia and hyperglycemia. Diabetes 2003;52:38–42
28. Finegood DT, Bergman RN, Vranic M. Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. Diabetes 1987;36:914–924
29. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 1959;82:420–430
30. Sikuler E, Polio J, Groszmann RJ, Hendler R. Glucagon and insulin metabolism in a portal-hypertensive rat model. Am J Physiol 1987;253:G110–G115
31. Ishida T, Chap Z, Chou J, et al. Effects of portal and peripheral venous insulin infusion on glucose production and utilization in depancreatized, conscious dogs. Diabetes 1984;33:984–990
32. Hovorka R, Jayatillake H, Rogatsky E, Tomuta V, Hovorka T, Stein DT. Calculating glucose fluxes during meal tolerance test: a new computational approach. Am J Physiol Endocrinol Metab 2007;293:E610–E619
33. Obici S, Feng Z, Arduini A, Conti R, Rossetti L. Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. Nat Med 2003;9:756–761
34. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009;55:611–622
35. Dube S, Errazuriz-Cruzat I, Basu A, Basu R. The forgotten role of glucose effectiveness in the regulation of glucose tolerance. Curr Diab Rep 2015;15:605
36. Pocai A, Obici S, Schwartz GJ, Rossetti L. A brain-liver circuit regulates glucose homeostasis. Cell Metab 2005;1:53–61
37. Inoue H, Ogawa W, Ozaki M, et al. Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism in vivo. Nat Med 2004;10:168–174
38. Inoue H. Central insulin-mediated regulation of hepatic glucose production [Review]. Endocr J 2016;63:1–7
39. Könner AC, Janoschek R, Plum L, et al. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metab 2007;5:438–449
40. Xiao C, Dash S, Stahel P, Lewis GF. Effects of intranasal insulin on endogenous glucose production in insulin-resistant men. Diabetes Obes Metab 2018;20:1751–1754
41. Gyte A, Pritchard LE, Jones HB, Brennand JC, White A. Reduced expression of the KATP channel subunit, Kir6.2, is associated with decreased expression of neuropeptide Y and agouti-related protein in the hypothalami of Zucker diabetic fatty rats. J Neuroendocrinol 2007;19:941–951
42. Burdakov D, Jensen LT, Alexopoulos H, et al. Tandem-pore K+ channels mediate inhibition of orexin neurons by glucose. Neuron 2006;50:711–722
43. Niki I, Ashcroft SJ. Characterization and solubilization of the sulphonylurea receptor in rat brain. Neuropharmacology 1993;32:951–957
44. Lahmann C, Kramer HB, Ashcroft FM. Systemic administration of glibenclamide fails to achieve therapeutic levels in the brain and cerebrospinal fluid of rodents [published correction appears in PLoS One 2019;14:e0215989]. PLoS One 2015;10:e0134476
45. Sola D, Rossi L, Schianca GP, et al. Sulfonylureas and their use in clinical practice. Arch Med Sci 2015;11:840–848
46. Koch L, Wunderlich FT, Seibler J, et al. Central insulin action regulates peripheral glucose and fat metabolism in mice. J Clin Invest 2008;118:2132–2147
47. Paranjape SA, Chan O, Zhu W, et al. Chronic reduction of insulin receptors in the ventromedial hypothalamus produces glucose intolerance and islet dysfunction in the absence of weight gain. Am J Physiol Endocrinol Metab 2011;301:E978–E983
48. Parton LE, Ye CP, Coppari R, et al. Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. Nature 2007;449:228–232
49. Brüning JC, Gautam D, Burks DJ, et al. Role of brain insulin receptor in control of body weight and reproduction. Science 2000;289:2122–2125
50. Campayo A, de Jonge P, Roy JF, et al.; ZARADEMP Project. Depressive disorder and incident diabetes mellitus: the effect of characteristics of depression. Am J Psychiatry 2010;167:580–588
51. de la Monte SM. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. Curr Alzheimer Res 2012;9:35–66
52. McIntyre RS, Soczynska JK, Konarski JZ, et al. Should depressive syndromes be reclassified as "metabolic syndrome type II"? Ann Clin Psychiatry 2007;19:257–264