Activation of $K_{\text{ATP}}$ channels suppresses glucose production in humans

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Increased endogenous glucose production (EGP) is a hallmark of type 2 diabetes mellitus. While there is evidence for central regulation of EGP by activation of hypothalamic ATP-sensitive potassium ($K_{\text{ATP}}$) channels in rodents, whether these central pathways contribute to regulation of EGP in humans remains to be determined. Here we present evidence for central nervous system regulation of EGP in humans that is consistent with complementary rodent studies. Oral administration of the $K_{\text{ATP}}$ channel activator diazoxide under fixed hormonal conditions substantially decreased EGP in nondiabetic humans and Sprague Dawley rats. In rats, comparable doses of oral diazoxide attained appreciable concentrations in the cerebrospinal fluid, and the effects of oral diazoxide were abolished by i.c.v. administration of the $K_{\text{ATP}}$ channel blocker glibenclamide. These results suggest that activation of hypothalamic $K_{\text{ATP}}$ channels may be an important regulator of EGP in humans and that this pathway could be a target for treatment of hyperglycemia in type 2 diabetes mellitus.

Introduction

Rodent studies have suggested that the central nervous system plays an important role in regulating energy and glucose homeostasis (1), such that glucose, insulin, and other nutrients (2) regulate endogenous glucose production (EGP) through activation of hypothalamic ATP-sensitive potassium ($K_{\text{ATP}}$) channels. $K_{\text{ATP}}$ channels are expressed in certain neuronal populations within the ventromedial hypothalamus as well as in the pituitary, muscle, and pancreatic β cells, in which they regulate insulin secretion (3). i.c.v. infusion of diazoxide, a $K_{\text{ATP}}$ channel agonist, decreased EGP within approximately 4 hours in rats (4). i.c.v. infusion of glibenclamide, a $K_{\text{ATP}}$ channel blocker, inhibited the effects of diazoxide and confirmed its central specificity. Recent studies in dogs have shown that the brain senses acute rises in insulin, subsequently impacting signaling events in the liver (5). Indeed, central insulin and nutrient signaling appear to regulate glucose metabolism via vagal efferents (4) and, ultimately, hepatic STAT3 activation (6). Since the brain is an obligate user of glucose, it would be of teleological advantage for the brain to play a role in EGP regulation.

Given potential species differences in regulation of EGP (5), it would be of critical importance to determine whether there is central regulation of EGP in humans. The availability of diazoxide for clinical use provides a means of activating $K_{\text{ATP}}$ channels in humans. Parallel studies in rodents and humans demonstrate that orally administered diazoxide crosses the blood-brain barrier (BBB) and suppresses EGP in a central, $K_{\text{ATP}}$-specific manner in rats and has suppressive effects on EGP in humans that are independent of pancreatic hormone secretion.

Results and Discussion

Diazoxide has extrapancreatic effects on EGP in humans. Ten non-diabetic human subjects received oral diazoxide or placebo 3 hours prior to a 4-hour euglycemic “pancreatic clamp” study, in which endogenous insulin was inhibited by somatostatin with replacement of basal insulin and glucoregulatory hormones (Figure 1A). This time course was established by earlier pilot studies (7). Diazoxide caused a decrease in EGP over the last 2 hours of the study, such that there was an approximately 30% decrease in EGP during the last hour of the clamp, 6–7 hours after oral dosing (diazoxide = 1.15 ± 0.13 mg/kg/min vs. placebo = 1.63 ± 0.17 mg/kg/min, $P$ = 0.02; Figure 1, B and C). There was no difference in rates of glucose uptake (diazoxide = 2.35 ± 0.26 mg/kg/min vs. placebo = 2.56 ± 0.26 mg/kg/min, $P$ = 0.24; Figure 1D). Deuterium enrichment of plasma glucose remained stable throughout the steady-state period in all studies.

In addition to activating $K_{\text{ATP}}$ channels in the brain, diazoxide has potent inhibitory effects on pancreatic insulin secretion (8). Thus, these pancreatic clamp conditions permitted the analysis of extrapancreatic effects of diazoxide. This single dose of diazoxide did not affect blood pressure, heart rate, or catecholamine levels but caused a small reduction in insulin levels at the onset of the clamp. C-peptide levels were comparably suppressed by somatostatin in diazoxide studies compared with those in versus placebo studies, and no differences were found in plasma levels of insulin, FFAs, cortisol, or glucagon in response to diazoxide compared to those in response to placebo during the clamps (Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI58035DS1). Given an anticipated portal/systemic insulin ratio of approximately 2.4:1 (9) and fasting systemic levels of insulin of approximately 10 μU/ml in our subjects, we would expect corresponding fasting portal levels of approximately 24 μU/ml. This was consistent with the systemic insulin levels observed during our clamp studies. Since peripheral vein insulin infusion in depankreazitated dogs resulted in portal insulin levels that approximated (within 20%) systemic insulin levels (10), it is likely that the liver was exposed to insulin levels similar to habitual fasting levels.

Although no studies have directly examined the effects of diazoxide on EGP in humans, a few observations suggest that diazoxide has beneficial effects on glucose metabolism, including improved insulin sensitivity (11). Furthermore, daily diazoxide administration for 6 months in subjects with newly diagnosed type 1 diabetes substanz-
tially improved glycemic control, despite having no effect on insulin secretion (12). In light of our findings, the observed improvement in glycemic control could be attributable to extrapancreatic effects of diazoxide, potentially mediated via the hypothalamus.

**Oral diazoxide crosses the BBB.** To establish the ability of oral diazoxide to cross the BBB, time course studies were performed in rats \((n = 13)\), in which cerebrospinal fluid (CSF) was sampled 1, 3, 5, or 6 hours after diazoxide gavage. CSF analysis indicated that oral diazoxide crossed the BBB fairly rapidly in rats, reaching CSF concentrations of \(0.26 ± 0.06 \, \mu \text{g/ml}\) 1 hour after gavage and a plateau of \(0.78 ± 0.03 \, \mu \text{g/ml}\) by the fourth hour (Supplemental Figure 1). These CSF diazoxide levels are consistent with those in previous studies in which i.c.v. diazoxide inhibited EGP in rats (4). Although not measured in the cited study, i.c.v. administration of 1.5 nmol diazoxide would likely have attained CSF diazoxide levels of approximately \(0.6 \, \mu \text{g/ml}\), given a CSF volume of approximately \(580 \, \mu \text{l}\) in a \(\sim 300 \, \text{g}\) rat (13). However, in the absence of studies comparing the ability of diazoxide to cross the BBB in humans compared with that in rats, we cannot exclude possible species differences.

**Diazoxide’s extrapancreatic effects on EGP are blocked by i.c.v. glibenclamide in rats.** To determine whether the above effects of oral diazoxide could be centrally mediated, we performed parallel studies in rats, in which we examined whether the effects of oral diazoxide on EGP and whether they could be abolished with i.c.v. glibenclamide. While oral diazoxide suppressed EGP by 54\% relative to saline control (diazoxide = \(2.23 ± 0.28 \, \text{mg/kg/min}\) vs. saline = \(4.85 ± 0.42 \, \text{mg/kg/min}\), \(P = 0.001\)), i.c.v. glibenclamide completely blocked these inhibitory effects (diazoxide plus glibenclamide = \(4.64 ± 0.41 \, \text{mg/kg/min}\), \(P = 0.72\) vs. saline; Figure 2C). These results, showing that the effects of oral diazoxide are blocked by i.c.v. glibenclamide infusion, suggest that oral diazoxide inhibits EGP via central K_{ATP}-dependent mechanisms. Furthermore, an upward trend in EGP with glibenclamide plus saline was not statistically significant (glibenclamide plus saline = \(5.87 ± 0.42 \, \text{mg/kg/min}\), \(P = 0.2\) vs. saline). While blocking central K_{ATP} channels should interrupt central regulation of EGP by hormones and nutrients, a substantial rise in EGP would be unlikely under these basal, euglycemic conditions. Of note, K_{ATP} channels are expressed in rat liver mitochondria (14), although their role in regulating glucose production is unknown. While the current studies demonstrated that inhibition of central K_{ATP} channels blocks the effects of systemic diazoxide, possible hepatic effect(s) of diazoxide cannot be excluded. Specific activity of \([3-^3\text{H}]-\text{glucose}\) was consistent throughout all studies, confirming steady-state conditions. Glucose uptake did not change among groups (\(P = 0.85\)). Clamped plasma glucose, insulin, glucagon, FFA, and C-peptide levels did not differ among groups (Supplemental Table 2).

**K_{ATP} channel activation suppresses hepatic gluconeogenic enzymes.** Since central K_{ATP} activation regulates EGP in concert with changes in gluconeogenic enzymes (4), gene expression and protein levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) were examined in rat liver after the clamps. There was a significant difference in Pepck gene expression between groups (\(P = 0.024\)), with decreased Pepck expression after diazoxide
treatment compared with that after saline treatment (relative gene expression, $0.04 \pm 0.0005$ vs. $0.32 \pm 0.007$, $P = 0.01$; Figure 3A). This effect was reversed by i.c.v. glibenclamide in diazoxide plus glibenclamide–treated rats (diazoxide plus glibenclamide $= 0.28 \pm 0.06$, $P = 0.9$ vs. saline). Similarly, a significant decrease in G6Pase gene expression with diazoxide was abolished by i.c.v. glibenclamide (diazoxide plus glibenclamide $= 1.31 \pm 0.06$ arbitrary units, $P = 0.2$ vs. saline). Similarly, there was a significant decrease in G6Pase protein levels with diazoxide that was abolished with glibenclamide (diazoxide $= 0.42 \pm 0.05$ arbitrary units vs. saline $= 0.67 \pm 0.07$ arbitrary units, $P = 0.03$; diazoxide plus glibenclamide $= 0.56 \pm 0.02$ arbitrary units, $P = 0.49$ vs. saline; Figure 3D).

$K_{ATP}$ channel activation increases hepatic STAT3 phosphorylation. Since central hormonal and nutritional signals increase liver STAT3 phosphorylation (p-STAT3), suppressing gluconeogenesis (15), we examined the impact of oral diazoxide with or without i.c.v. glibenclamide on hepatic p-STAT3. After these studies, there was a difference in p-STAT3 among the groups ($P = 0.001$), with a significant increase in diazoxide group compared with the saline group ($0.42 \pm 0.03$ vs. $0.27 \pm 0.02$ arbitrary units, $P = 0.004$), which was reversed by i.c.v. glibenclamide in diazoxide plus glibenclamide group ($0.27 \pm 0.03$ arbitrary units; $P = 1.0$ vs. saline) (Figure 2D). To exclude inadvertent differences in direct hepatic insulin signaling, we measured phosphorylated Akt (pAkt) in rat liver after the clamp studies and did not observe any significant effects of oral diazoxide, i.c.v. glibenclamide, or the combination on hepatic pAkt (diazoxide $= 0.64 \pm 0.17$ arbitrary units, saline $= 0.48 \pm 0.10$ arbitrary units, diazoxide plus glibenclamide $= 0.73 \pm 0.14$ arbitrary units, saline plus glibenclamide $= 0.44 \pm 0.05$ arbitrary units, $P = 0.37$ for 4 groups).
peptide and proproiomelanocortin neurons in the ventromedial hypothalamus affects suppression of EGP and whole-body glucose homeostasis (24, 25). Ultimately, EGP is likely to be regulated by both central and peripheral mechanisms (26), and the importance of central effects of glucose, insulin, and FFA on EGP should be considered relative to the direct effects of these factors on the liver.

In summary, to our knowledge these are the first studies in humans examining the possibility of central regulation of EGP. While extrapancreatic KATP channel activation suppresses EGP in humans, complementary rodent studies suggest that this regulation is mediated by hypothalamic KATP channels. Given accumulating evidence for the importance of central signaling in regulating EGP, modulating this pathway could provide a novel therapeutic approach for treating diabetes.

**Methods**

*Human subjects.* Ten healthy nondiabetic subjects (7 males; age, 33.0 ± 2.5 years; BMI, 27.4 ± 1.5 kg/m²), who were taking no medications and had no family history of diabetes, were recruited. Additional information on the human studies, rat studies, Western blot, and RT-PCR can be found in the Supplemental Methods.

*Human infusion studies.* Four-hour euglycemic pancreatic clamp studies (*t* = 0 to *t* = 240 minutes) were performed, as previously described (Figure 1A) (27). Three hours before onset of the clamp study, the subjects received either oral diazoxide 4 mg/kg or matched placebo in a randomized, double-blinded fashion.

*Rat studies.* Five- to seven-week-old male Sprague Dawley rats (*n* = 22) (Charles River Laboratories), with an average weight of 313.8 ± 2.3 g, were studied under the following conditions: (a) oral (gavage) saline control (*n* = 6); (b) oral (gavage) diazoxide (*n* = 6); (c) oral (gavage) diazoxide with i.c.v. infusion of KATP channel blocker glibenclamide (*n* = 5); and (d) oral (gavage) saline with i.c.v. glibenclamide (*n* = 5). 120 minutes prior to the infusion studies, rats were anesthetized, and either saline or diazoxide (100 mg/kg) was administered by oral gavage (Figure 2A). An i.c.v. infusion of glibenclamide (0.006 µl/min; *n* = 5 rats) or saline was started at *t* = –120 minutes. At *t* = 0 minutes, a primed continuous i.v. infusion of [3-H]-glucose was begun and maintained for 4 hours to assess glucose kinetics. A peripheral basal insulin euglycemic clamp was performed for the final 2 hours of the infusion study (*t* = 120–240 minutes), as previously described (22).

*Western blot analysis.* Western blotting was performed on rat liver tissue to quantify p-STAT3, total STAT3, pAkt, total Akt, PEPCK, and G6Pase. Real-time RT-PCR. Real-time RT-PCR was performed to examine the expression of PEPCK and G6Pase in rat liver, as previously described (19) (Supplemental Table 3).

*Human plasma hormone and substrate determinations.* Plasma glucose, insulin, C-peptide, FFA, and glucagon levels were measured as previously described (19). Plasma cortisol and catecholamine levels were measured by radioimmunoassay (28) (Immunobiological Laboratories America).

*Statistics.* Paired Student’s *t* tests (2 tailed) were used to compare diazoxide and placebo studies in humans. One-way ANOVA was used to compare results among groups in rats, with subsequent use of Tukey and Scheffe
tests for between-group comparisons when significant differences were noted. For all analyses, a P value of less than 0.05 was considered significant. All data are presented as mean ± SEM.

Study approval. All procedures were approved by the Institutional Review Board of Albert Einstein College of Medicine. All animal studies were approved by the Institutional Animal Care and Use Committee of Albert Einstein College of Medicine.

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