JCI The Journal of Clinical Investigation

**Amendment history:**
- Erratum (June 2012)

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

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*J Clin Invest.* 2011;121(12):4916-4920. https://doi.org/10.1172/JCI58035.

**Brief Report**

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 ($K_{ATP}$) 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 $K_{ATP}$ 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 $K_{ATP}$ channel blocker glibenclamide. These results suggest that activation of hypothalamic $K_{ATP}$ 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.



# Activation of $K_{ATP}$ 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 ($K_{ATP}$) 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 $K_{ATP}$ 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 $K_{ATP}$ channel blocker glibenclamide. These results suggest that activation of hypothalamic $K_{ATP}$ 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 ($K_{ATP}$) channels. $K_{ATP}$ 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 $K_{ATP}$ channel agonist, decreased EGP within approximately 4 hours in rats (4). i.c.v. infusion of glibenclamide, a $K_{ATP}$ 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 $K_{ATP}$ 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, $K_{ATP}$-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 $K_{ATP}$ 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 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 depancreatized 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 substan-

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Citation for this article:** *J Clin Invest.* 2011;121(12):4916–4920. doi:10.1172/JCI58035.

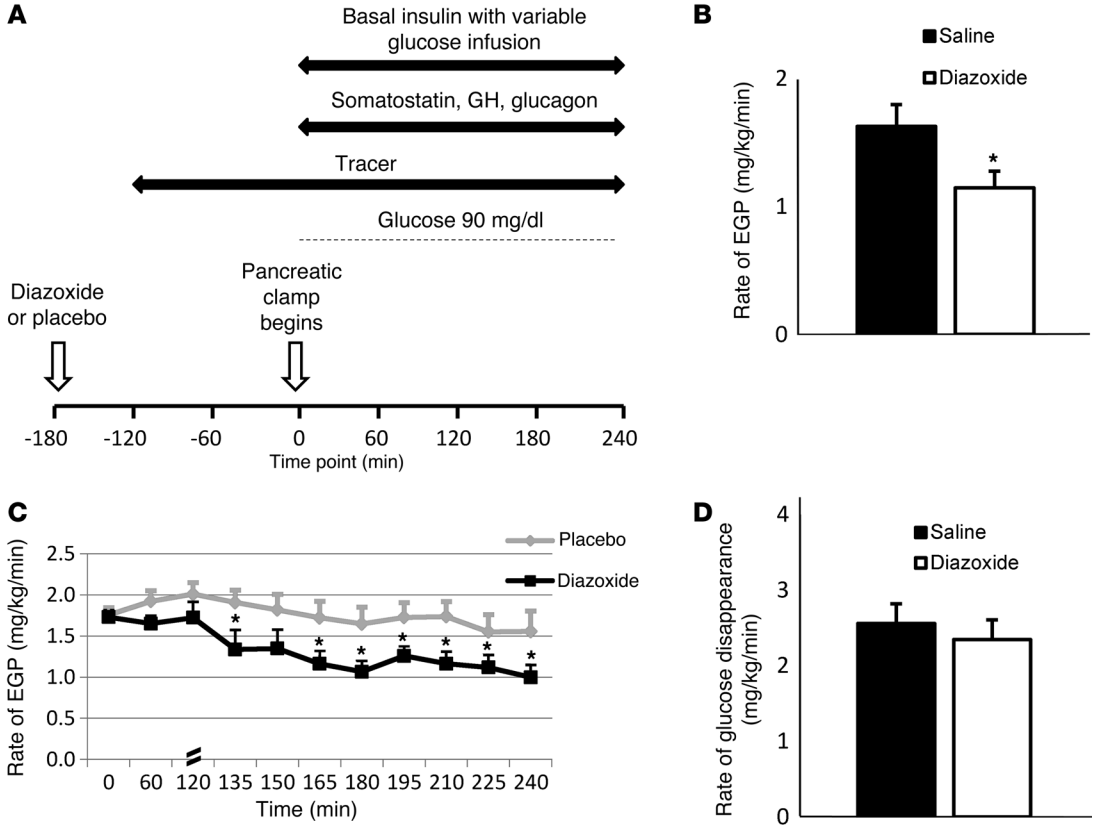


**Figure 1**
Effects of $K_{ATP}$ channel activation on EGP in humans. (**A**) Protocol scheme for human clamp studies. (**B**) EGP during final hour of clamps (*$P$ = 0.02). (**C**) Time course of EGP during clamp studies. *$P$ < 0.05. (**D**) Rate of glucose disappearance during final hour of clamps. Data represent mean ± SEM.

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 μg/ml 1 hour after gavage and a plateau of 0.78 ± 0.03 μg/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 μg/ml, given a CSF volume of approximately 580 μl in a ~300 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 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 mg/kg/min vs. saline = 4.85 ± 0.42 mg/kg/min, $P$ = 0.001), i.c.v. glibenclamide completely blocked these inhibitory effects (diazoxide plus glibenclamide = 4.64 ± 0.41 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 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-$^3$H]-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

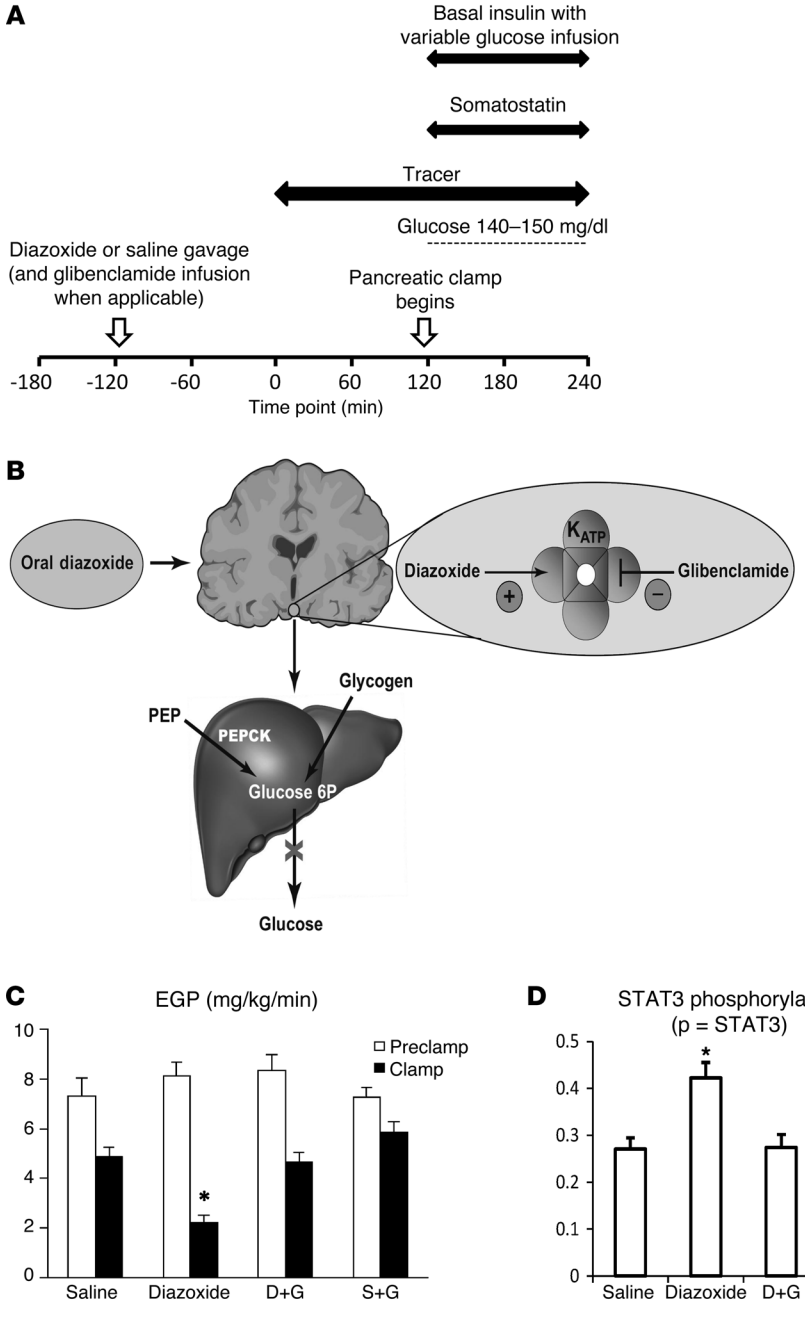


**Figure 2**
Effects of $K_{ATP}$ channel activation on EGP in rodents. (**A**) Protocol scheme for rat clamp studies. (**B**) Outline of rationale for oral/i.c.v. studies. Experimental design was as follows: oral diazoxide was administered under pancreatic clamp conditions to examine its effects on EGP and gluconeogenic enzymes (PEPCK and G6Pase). Glibenclamide was administered i.c.v. to block hypothalamic KATP channels, thereby determining whether oral diazoxide exerts its effects centrally. PEP, phosphoenolpyruvate, a gluconeogenic substrate. (**C**) EGP during the preclamp phase (60–120 minutes) and final hour of the study (180–240 minutes). D+G, diazoxide plus glibenclamide; S+G, saline plus glibenclamide. **P* = 0.001 for saline versus diazoxide. (**D**) Hepatic p-STAT3. G+S, glibenclamide plus saline. **P* = 0.004 for saline versus diazoxide. Data represent mean ± SEM.

treatment compared with that after saline treatment (relative gene expression, 0.04 ± 0.0005 vs. 0.32 ± 0.007, *P* = 0.01; Figure 3A). This effect was reversed by i.c.v. glibenclamide in diazoxide plus glibenclamide–treated rats (relative *Pepck* expression, diazoxide plus glibenclamide = 0.28 ± 0.06, *P* = 0.9 vs. saline). Similarly, a significant decrease in *G6Pase* gene expression with diazoxide was abolished by i.c.v. glibenclamide (relative gene expression, diazoxide = 0.01 ± 0.004 vs. saline = 0.03 ± 0.007, *P* = 0.02; diazoxide plus glibenclamide = 0.03 ± 0.003, *P* = 0.49 vs. saline; Figure 3B).

Additionally, hepatic PEPCK and G6Pase protein levels were examined by Western blotting and paralleled the changes seen in gene expression. PEPCK protein levels were significantly different among groups (*P* = 0.002), with decreased PEPCK in diazoxide-treated rats compared with that in saline-treated rats (0.98 ± 0.05 arbitrary units vs. 1.53 ± 0.13 arbitrary units, *P* = 0.01; Figure 3C). This effect was reversed when the central effects of diazoxide were blocked by glibenclamide (diazoxide plus glibenclamide = 1.31 ± 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 ± 0.05 arbitrary units vs. saline = 0.67 ± 0.07 arbitrary units, *P* = 0.03; diazoxide plus glibenclamide = 0.56 ± 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 ± 0.03 vs. 0.27 ± 0.02 arbitrary units, *P* = 0.004), which was reversed by i.c.v. glibenclamide in diazoxide plus glibenclamide group (0.27 ± 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 ± 0.17 arbitrary units, saline = 0.48 ± 0.10 arbitrary units, diazoxide plus glibenclamide = 0.73 ± 0.14 arbitrary units, saline plus glibenclamide = 0.44 ± 0.05 arbitrary units, *P* = 0.37 for 4 groups).

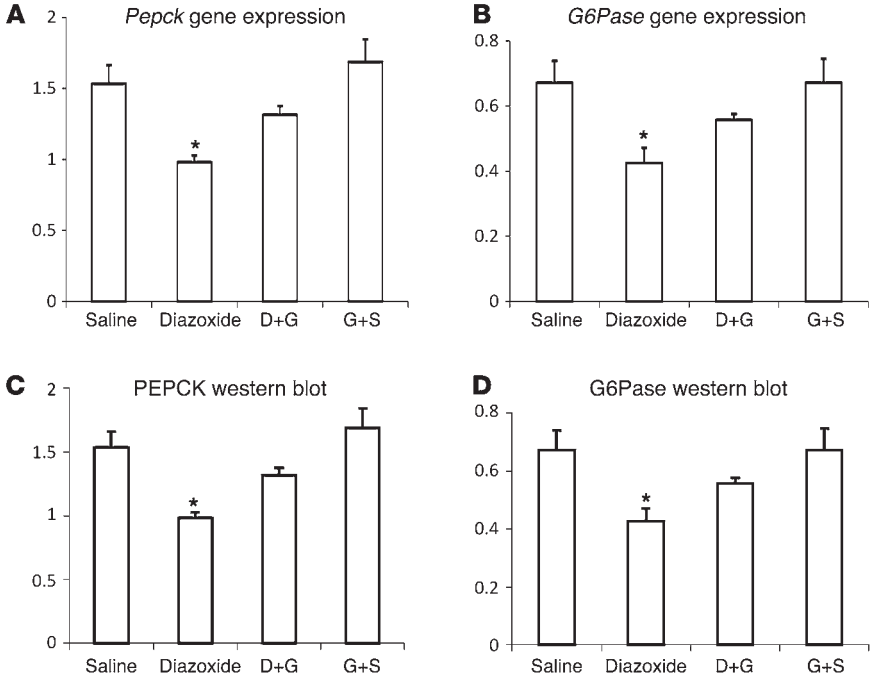


**Figure 3**
Gene expression and protein concentrations of PEPCK and G6Pase in rat liver. (**A**) Levels of *Pepck* expression. **P* = 0.01 for saline versus diazoxide. (**B**) Levels of *G6Pase* expression. **P* = 0.02 for saline versus diazoxide. (**C**) Levels of PEPCK expression. **P* = 0.01 for saline versus diazoxide. (**D**) Levels of G6Pase expression. **P* = 0.03 for saline versus diazoxide. Data represent mean ± SEM.

*Time course of diazoxide effects*. Maximal effects of oral diazoxide on EGP were observed at approximately 6 to 7 hours in humans, with significant decreases in EGP by 315 minutes. Factors contributing to this time course probably included the time required for central appearance of oral diazoxide (maximal by 4 hours in rats) and the effects of central signals on hepatic p-STAT3 and subsequently on gluconeogenic enzyme transcription and translation (16). This is consistent with the observation that raising carotid and vertebral artery insulin levels for 3 hours did not affect EGP in dogs (17). Moreover, intraportal insulin infusion caused rapid (45%–50% by 30 minutes), followed by marked (80%–90% by 240 minutes), decreases in hepatic PEPCK and G6Pase expression, with significant increases in hepatic p-STAT3 by 240 minutes (18). Collectively, this suggests that rapid, direct effects and more delayed central effects are likely to contribute to the regulation of hepatic glucose fluxes. Indeed, the fact that approximately 5 hours of continuous hyperinsulinemia are required for maximal effects of insulin on whole-body glucose metabolism (19), despite maximal effects on tissue insulin signaling within a few minutes (20), may highlight the physiologic relevance of these more delayed central effects. High-fat feeding for 3 days attenuates the central control of hepatic glucose homeostasis (21), suggesting that central pathways integrate longer-term signals in regulating glucose homeostasis, as opposed to more acutely responsive peripheral mechanisms. Additionally, the importance of central regulation of glucose metabolism is highlighted by the phenotypes of several transgenic models. Inducible inactivation of brain insulin receptor expression caused hyperglycemia in mice (22). Specifically, knockdown of insulin receptors in the ventromedial hypothalamus caused hepatic insulin resistance and glucose intolerance (23). Manipulating agouti-related peptide and proopiomelanocortin 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 $K_{ATP}$ channel activation suppresses EGP in humans, complementary rodent studies suggest that this regulation is mediated by hypothalamic $K_{ATP}$ 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/m$^2$), 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 $K_{ATP}$ 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-$^3$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.

## Acknowledgments

The authors thank Luciano Rossetti, Do-Eun Lee, Maria Martin, Daniel Stein, Nancy Brown, Jeff Petro, Bing Liu, Laura Clintoc, Marisa Morrill, and Stephanie Lawrence for their valuable assistance. This work was supported by the National Institutes of Health (DK 20541, DK048321, DK069861, DK45024, K23 RR023335, and P01-AG021654), the American Diabetes Association, and CTSA grants UL1RR025750, KL2RR025749, and TL1RR025748.

Received for publication March 15, 2011, and accepted in revised form October 5, 2011.

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