Priya S. George,[1] Roger Tavendale,[2] Colin N.A. Palmer,[2] and Rory J. McCrimmon[1]

Diazoxide Improves Hormonal Counterregulatory Responses to Acute Hypoglycemia in Long-standing Type 1 Diabetes

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COMPLICATIONS

Individuals with long-standing type 1 diabetes (T1D) are at increased risk of severe hypoglycemia secondary to impairments in normal glucose counterregulatory responses (CRRs). Strategies to prevent hypoglycemia are often ineffective, highlighting the need for novel therapies. ATP-sensitive potassium (K_{ATP}) channels within the hypothalamus are thought to be integral to hypoglycemia detection and initiation of CRRs; however, to date this has not been confirmed in human subjects. In this study, we examined whether the K_{ATP} channel-activator diazoxide was able to amplify the CRR to hypoglycemia in T1D subjects with long-duration diabetes. A randomized, double-blind, placebo-controlled cross-over trial using a stepped hyperinsulinemic hypoglycemia clamp was performed in 12 T1D subjects with prior ingestion of diazoxide (7 mg/kg) or placebo. Diazoxide resulted in a 37% increase in plasma levels of epinephrine and a 44% increase in plasma norepinephrine during hypoglycemia compared with placebo. In addition, a subgroup analysis revealed that the response to oral diazoxide was blunted in participants with E23K polymorphism in the K_{ATP} channel. This study has therefore shown for the first time the potential utility of K_{ATP} channel activators to improve CRRs to hypoglycemia in individuals with T1D and, moreover, that it may be possible to stratify therapeutic approaches by genotype.

The goal of insulin therapy in type 1 diabetes (T1D) is ultimately to restore glucose levels to the nondiabetic physiological range to prevent the development of micro- and macroangiopathy. Intensive insulin therapy (IIT), using multi-injection regimens or continuous subcutaneous insulin infusion, goes some way toward achieving this but is associated with a rapidly increasing rate of severe hypoglycemia as glycemic targets are achieved (1). The high rates of severe hypoglycemia with IIT reflect the limitations of current insulin replacement therapy as well as the presence in almost all individuals with T1D of defects in the normal homeostatic (counterregulatory) response to hypoglycemia (2). Defective counterregulation results primarily from a failure to suppress endogenous insulin secretion, an inability to stimulate α-cell glucagon release, and suppression of the catecholaminergic response to hypoglycemia (2). The latter defect is associated with impaired awareness of hypoglycemia (IAH), and together they markedly increase (25-fold) an individual's risk of severe hypoglycemia (3). Given that dysregulation of α-cell glucagon release is thought to result mainly from an intraislet defect secondary to β-cell destruction (2), research efforts have focused on understanding the mechanisms that contribute to the suppression of catecholaminergic and symptom responses to hypoglycemia in T1D in the hope that this would lead to novel therapeutic strategies or interventions.

The seminal work by Heller and Cryer (4) established that even a single episode of hypoglycemia resulted in reduced symptom and catecholaminergic counterregulatory responses (CRRs) during an equivalent episode of hypoglycemia induced within 24 h. Recurrent exposure

[1]Division of Diabetes and Cardiovascular Medicine, Medical Research Institute, Ninewells Hospital and Medical School, Dundee, Scotland, U.K.
[2]The Pat McPherson Centre for Pharmacogenomics & Pharmacogenetics, Medical Research Institute, Ninewells Hospital and Medical School, Dundee, Scotland, U.K.
Corresponding author: Priya S. George, p.george@dundee.ac.uk.
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to hypoglycemia is thought to therefore underlie the pronounced suppression of symptom and catecholaminergic responses seen after IIT (5), a hypothesis supported by the observation in animals of greater suppression of hypoglycemic counterregulation after more frequent hypoglycemia (6) and, conversely, that strict avoidance of hypoglycemia can at least partly restore symptom and catecholaminergic responses to hypoglycemia in T1D (7). Unfortunately, hypoglycemia avoidance in clinical practice is difficult to achieve, and despite the positive benefits seen in randomized controlled trials from structured educational programs (8,9), continuous glucose monitoring (10), and sensor-augmented strategies (11), the frequency of severe hypoglycemia in routine clinical practice has remained relatively unchanged in the last 2 decades (12,13). This highlights the need for novel approaches to the prevention of severe hypoglycemia and restoration of IAH.

One potential target for therapeutic intervention is the ATP-sensitive potassium (K_{ATP}) channel. The K_{ATP} channel is a ligand-gated ion channel composed of four inward-rectifier potassium ion channels and four sulfonylurea receptor subunits (SUR-1, SUR 2-A, and SUR 2-B) and plays a critical role in transducing changes in cellular energy status into changes in action potential firing. Glucose-sensing hypothalamic neurons important to the detection of hypoglycemia express SUR-1–containing K_{ATP} channels (14), and mice lacking functional K_{ATP} channels display abnormal glucose sensing (15). In rats, direct in vivo local application of SUR-1 activators to the ventromedial hypothalamus (VMH) amplifies (16), whereas local SUR-1 inhibition suppresses (17), the CRR to acute hypoglycemia. Moreover, systemic delivery of a SUR-1–selective activator amplified CRRs during hyperinsulinemic-hypoglycemic clamp studies in normal and recurrently hypoglycemic rodents, an effect that could be reversed by VMH K_{ATP} channel inhibition (16,18).

Although these studies in animal models provide robust support for a role for K_{ATP} channels in the detection of hypoglycemia, it has not yet been convincingly shown that K_{ATP} channels are also important to the detection of hypoglycemia in human subjects and therefore a potential target for therapeutic intervention. Thus, this study was designed to specifically test the hypothesis that K_{ATP} channels were also integral to the detection of hypoglycemia in individuals with established T1D.

RESEARCH DESIGN AND METHODS

This was a single-center, double-blinded, placebo-controlled randomized controlled trial. Ethical approval was obtained from an independent research ethics committee and the Medicines Healthcare Products Regulatory Agency (MHRA). The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants before inclusion in the study. Inclusion criteria were diagnosis of T1D, age of 18 to 55 years, tightly controlled glycemia (HbA_{1c} <64 mmol/L or 8% Diabetes Control and Complications Trial [DCCT]), and greater than 5 years' disease duration. Reasons for exclusion were history of significant cardiac, hepatic, renal, or neurological disease; being a pregnant or breast-feeding mother; and already taking any medications that may interact with diazoxide. All participants were identified using the Scottish Diabetes Research Network (SDRN), and the study took place at the Clinical Research Centre, Ninewells Hospital, Dundee.

After the initial screening, which included collection of demographic information, each subject attended the clinical research center on four occasions. On two of these visits, separated by at least 2 weeks, the subject was given oral diazoxide or placebo before undergoing a hyperinsulinemic-hypoglycemic clamp study. Diazoxide and placebo were given in capsules that were identical in appearance and supplied by independent pharmacists according to a computer-generated randomization. The participants and investigators were both blinded to allocation of treatment. The other two visits were to fit each participant with a continuous glucose monitor (RT-CGM), with low-glucose suspend (set at 4.5 mmol/L) where applicable, for 48 h before each clamp study, to ensure the absence of significant hypoglycemia before the clamp procedure.

Experimental Hypoglycemia

The evening before attendance, each participant was advised to reduce his or her nighttime long-acting insulin by ~20% and to fast for at least 8 h before coming to the Clinical Research Centre at 0800 h. On the morning of the clamp, a cannula was inserted into a dorsal hand vein of the nondominant hand in a retrograde fashion and then placed in a heated box at 55°C to arterialize venous blood (19). This catheter was used for blood sampling during the clamp study. In the contralateral arm, the antecubital vein was cannulated for insulin and glucose infusions.

Participants were given oral diazoxide (7 mg/kg) or placebo 2 h before the start of the euglycemic plateau. The timing of oral diazoxide ingestion was based on the available literature indicating that its hypotensive and antihypoglycemic effect lasted ~3–12 h, with a peak action at ~5 h (20). Participants were subsequently started on insulin at 50 mL/h for priming purposes until the blood glucose dropped to below 7 mmol/L, after which a rate of 1.5 mU/kg/min was maintained for the duration of the clamp. A variable 20% dextrose infusion (Infusomat Space; Braun) was adjusted every 5 min based on bedside glucose measures. Euglycemia (glucose 4–5 mmol/L) was achieved and maintained during the first 2 h of the clamps, and subsequently, blood glucose was reduced by 0.5 mmol/L every 40 min to a final glucose level of 2.5 mmol/L. This was maintained for 40 min before glucose levels were allowed to return to the euglycemic range.

Physiological Measurements

Blood pressure and pulse rate were measured every 10 min using an Accutor Plus Monitor (Datascope Corp., Mahwah, NJ).

Counterregulatory Hormones

Arterialized blood for insulin and counterregulatory hormones (epinephrine, norepinephrine, glucagon) was taken at midpoint and at the end of each plateau.

Symptoms

Subjects rated symptoms at the midpoint of every glucose plateau. Symptoms were scored on a validated questionnaire, the Edinburgh Hypoglycemia Scale (21), scoring from 1 (not at all) to 7 (very severe) on a visual analog scale.

Cognitive Function Tests

A battery of psychometric tests known to be sensitive to hypoglycemia were applied in the same order, starting at the midpoint of each plateau: Trail Making B (TMB) (22), Digit Span Backward (Dig-B) (23), Digit Symbol Substitution Test (DSS) (24), and Four Choice Reaction Time (4CRT) (25).

Laboratory Assays

Whole blood was measured at the bedside by a glucose oxidase method (Analox GM9D; Analox Instruments, London, U.K.). Samples were centrifuged within 2 h to separate the plasma and stored at −80°C before assay. Hormone levels of insulin (RIA, DiaSorin; coefficient of variation [CV] inter −6.7%, intra −5.8%), glucagon (RIA, Millipore UK; CV inter 4.9%, intra 8.8%), and epinephrine (EIA, Alpco; CV inter 22%, intra 16%), norepinephrine (EIA, Alpco; CV inter 16%, intra 22%) were measured by ELISA, and samples were analyzed in duplicate according to the manufacturer's instructions. Genomic DNA was prepared from whole blood using an Autopure DNA preparation robot (Qiagen). Genotyping of rs5219 was performed by TaqMan-based allelic discrimination (Thermo-Life Technologies) according to the manufacturer's instructions.

Data and Statistical Analysis

The prespecified primary end point was the magnitude of epinephrine responses at a glucose level of 2.5 mmol/L. Secondary outcomes examined whether oral diazoxide would affect glucose thresholds, defined using published protocols (26,27), for activation of hormonal, symptomatic, and cognitive responses or result in significant changes in heart rate or blood pressure. Data are presented as mean (SE). For the primary end point, normally distributed data were compared using paired-samples *t* tests, and nonnormally distributed data were compared using the Wilcoxon signed rank test. $P < 0.05$ was considered statistically significant. Repeated measures ANOVA was used to determine differences in other parameters measured over time, with *t* testing used to localize effects where indicated. Statistical analyses were conducted using GraphPad Prism 6 software.

RESULTS

Participant Characteristics

Recruitment was from January 2012 to September 2012. Of the 24 participants screened, 6 did not meet the inclusion criteria and 4 withdrew consent; 14 subjects were randomized, and 2 subsequently withdrew after the first clamp study. Twelve participants (6 male and 6 female) completed all stages of the study (two clamps). This group had a median age of 43 (range 18–52). Median (range) duration of diabetes was 24 (6–40) years, and median HbA_{1c} was 7.6%/60 mmol/mol (6.9–8%/52–64 mmol/mol). An equal number of subjects received multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) therapy. Five of 12 participants had IAH as classified by the Gold criteria (≥4) (28).

Hyperinsulinemic-Hypoglycemic Clamp Studies

Mean (SEM) baseline blood glucose levels before ingestion of diazoxide (D) and placebo (P) did not differ significantly between the two study days (10.6 [0.7] vs. 11.8 [1.0], D vs. P; $P = 0.90$). Glucose levels during the two clamp procedures were also well matched (Fig. 1). As expected, plasma glucose dropped with time over the stepped clamp (main effect of time $F_{16,187} = 37.60$, $P < 0.05$). This drop was comparable in the two treatment groups (main effect of treatment $F_{1,187} = 0.2882$, $P = 0.59$), with no time × treatment interaction (time × treatment $F_{16,187} = 0.4403$, $P = 0.97$). We maintained a mean insulin level of 79 (3.0) versus 76 (2.8) mU/L (D vs. P) throughout the clamp period (P = NS).

K_{ATP} Channel Activation With Diazoxide Amplifies the CRR to Hypoglycemia

We found that after oral administration of diazoxide, there was a 37% increase in the prespecified primary outcome mean (SEM) epinephrine responses (0.40 [0.06] vs. 0.29 [0.05] ng/mL, D vs. P; $P < 0.05$) and a 44% increase in mean (SEM) norepinephrine (0.85 [0.07] vs. 0.59 [0.06] ng/mL, D vs. P; $P < 0.05$) at plasma glucose of 2.5 mmol/L (Fig. 2*A* and *B*). Glucagon levels remained, as expected, suppressed during hypoglycemia, with no significant differences found between groups (57.8 [11] vs. 50.0 (7.1) ng/L, D vs. P; $P = 0.21$). Consistent with the amplified catecholaminergic response to hypoglycemia, the glucose infusion rates required to maintain the hypoglycemic plateau were significantly lower at 2.5 mmol/L after oral diazoxide (71.6 ± 1.8 vs. 77.5 ± 2.1, D vs. P; $P < 0.05$).

Despite the improved counterregulatory hormone response to hypoglycemia, participants experienced similar total symptom scores at an arterialized plasma glucose of 2.5 mmol/L after administration of diazoxide (22 [3] vs. 19 [3], D vs. P; $P = 0.32$). Similarly, the overall increase in autonomic symptoms after diazoxide was not significant (10 [1] vs. 9 [1]; $P = 0.26$). Cognitive performance of participants during the 2.5 mmol/L step was mixed, with no significant effect of diazoxide on TMB (30 [4] vs. 33 [5] s, D vs. P; $P = 0.65$), Dig-B (6 [1] vs. 7 [1], D vs. P; $P = 0.38$), or 4CRT (547 [21] vs. 543 [18], D vs. P; $P = 0.82$), and a significant deterioration in DSS after diazoxide (70 [9] vs. 81 [8]; D vs. P; $P < 0.05$; Supplementary Fig. 1*A*–*C*).

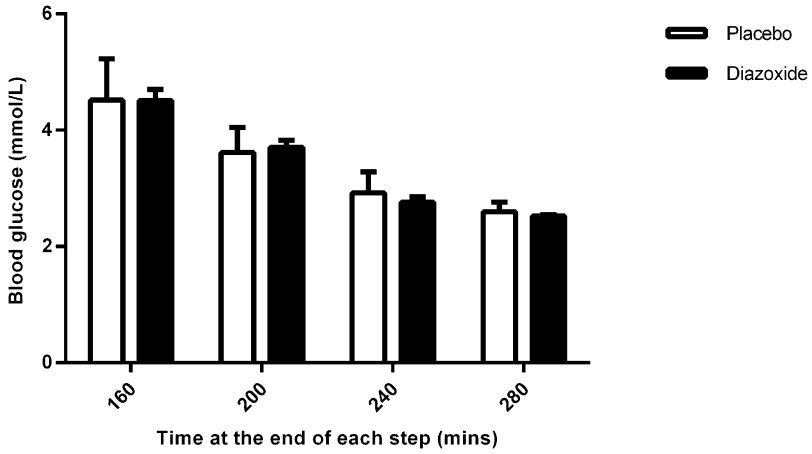

Figure 1—Glucose profiles during stepped hyperinsulinemic-hypoglycemic clamp studies. The hyperinsulinemic-hypoglycemic clamp technique was used to slowly drop the blood glucose from euglycemia (4.0 mmol/L) to hypoglycemia (3.5, 3.0, 2.5 mmol/L). The drug (diazoxide or placebo) was given at 0 min. After 120 min, blood glucose levels were slowly dropped to euglycemia during that time, after which the clamp was commenced. Each nadir was achieved over 20 min, and then maintained for 20 min. The average blood glucose level achieved at each of the desired steps during the diazoxide and placebo clamp studies is shown in the bar chart.

K_{ATP} Channel Activation With Diazoxide Did Not Lower Thresholds for CRRs to Hypoglycemia

Secondary outcomes in this study were to determine whether diazoxide would lower (higher glucose for initiation) the glucose thresholds for onset of hormone responses. Thresholds were defined as the time of onset of a sustained (two or more successive time points) increase in hormone concentrations $\geq$2 SDs above the mean of the two baseline measurements for that hormone. The glucose thresholds for generation of epinephrine and norepinephrine responses to hypoglycemia were both lower after administration of diazoxide, but the difference did not reach statistical significance (Supplementary Table 1).

The E23K Polymorphism in K_{ATP} Channels Predicts Response to Diazoxide During Hypoglycemia

The E23K polymorphism in the K_{ATP} channel results in an increase in the likelihood of the K_{ATP} channel being open in the resting state (29,30) and influences individual responses to sulfonylureas (31). To determine whether the E23K polymorphism might influence individual responses to diazoxide, we genotyped the 12 participants in the study and divided the cohort into diazoxide responders and diazoxide nonresponders. A diazoxide responder was defined as an individual who had a greater than double the SEM increase in epinephrine response at 2.5 mmol/L after diazoxide. In our study cohort, 7 of the 12 participants (58%) carried the K23 allele (2-KK, 5-EK), and the rest carried wild-type homozygous E23. Intriguingly, participants who expressed only the wild-type E23 allele were all diazoxide responders, whereas those hetero- or homozygous for the K23 allele were significantly less likely to respond to diazoxide (Pearson χ^2 = 6.12, P = 0.013; Fig. 3*A* and *B*). Those who expressed the wild-type E23 allele also showed a greater magnitude of epinephrine response, particularly as the blood glucose dropped to 2.5 mmol/L (Supplementary Fig. 3).

Adverse Events

Systolic blood pressure was comparable between the two groups, with no effect of treatment (main effect of treatment $F_{1,22}$ = 0.001228, P = 0.97). Similarly, there was no effect of treatment on diastolic blood pressure (main effect of treatment $F_{1,22}$ = 0.4602, P = 0.50) or on pulse rate (main effect of treatment $F_{1,22}$ = 2.893, P = 0.10) (Supplementary Fig. 2*A–C*).

One participant had short-lived nausea and vomiting in the recovery phase of both D and P studies and reported nausea as being one of her usual symptoms during hypoglycemia. One participant had nausea and a bout of vomiting in the recovery stage after receiving diazoxide. There were no serious adverse events or reactions.

DISCUSSION

It is now generally accepted that the brain, and particularly specialized neuronal populations within the hypothalamus, plays a major role in both the detection of hypoglycemia and the development of IAH (2). The importance of K_{ATP} channels to hypothalamic glucose sensing was first proposed by Mayer (32), and the critical role of K_{ATP} channels in hypothalamic glucose sensing has since been demonstrated in cell culture models (33), ex vivo hypothalamic slices (14), transgenic mouse models (15), and rodent in vivo pharmacological studies (16–18). In the current study, we now extend these findings by demonstrating for the first time in individuals with T1D of long duration that oral diazoxide (7 mg/kg) given before acute hypoglycemia can significantly increase the magnitude of epinephrine and norepinephrine CRRs. Moreover, we make the novel observation that the E23K polymorphism in the Kir6.2 subunit of the K_{ATP} channel predicts response to diazoxide therapy during hypoglycemia in T1D.

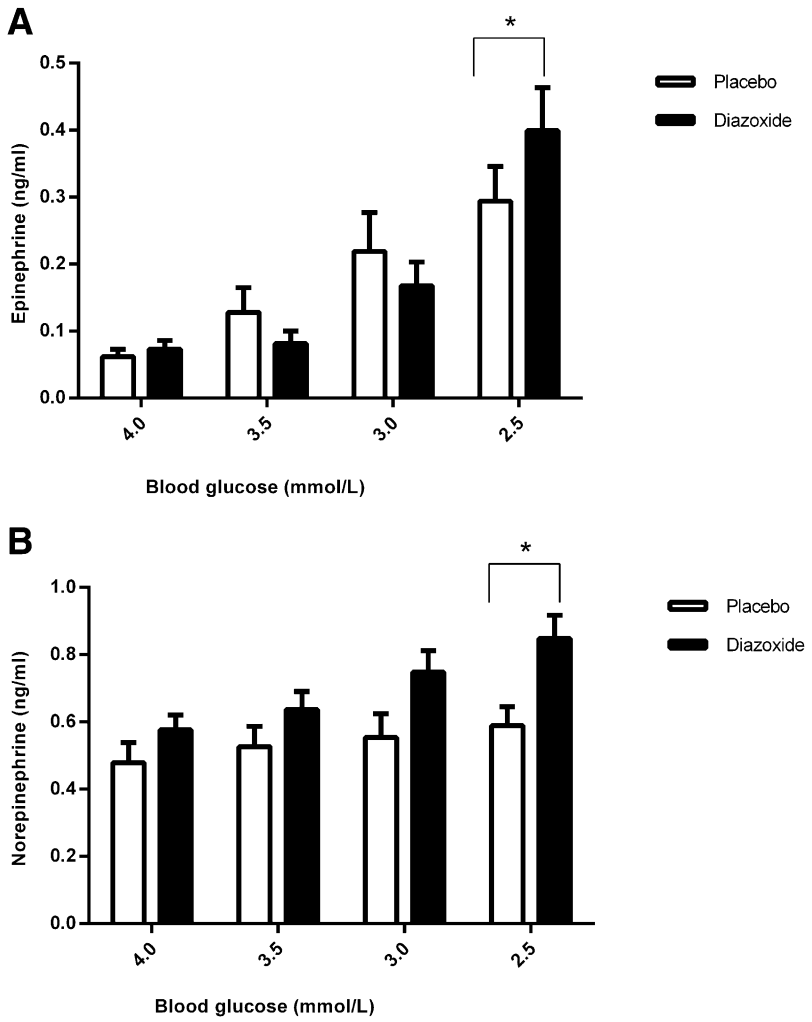

Figure 2—Diazoxide amplifies CCRs during acute hypoglycemia in long-standing T1D. Plasma epinephrine (*A*) and norepinephrine (*B*) levels are shown during baseline and at each hypoglycemic plateau. Values are shown as mean (SEM). *$P < 0.05$.

The mechanism by which diazoxide improves the neuroendocrine response remains unclear. The in vitro and in vivo rodent literature support a central action of K_{ATP} activators; however, all glucose-sensing cells, both centrally and peripherally, have been shown to contain Kir6.2 and SUR-1 components of the K_{ATP} channel. It is therefore possible that oral diazoxide has acted primarily through peripheral K_{ATP} channels, such as those in the hepatoportal veins (34). The comparatively "slow-fall" in glucose with the multistep clamp would also be consistent with activation through hepatoportal sensors (34). In contrast, studies of direct hypothalamic modulation of K_{ATP} channels in rodents during hypoglycemia (16,17) support a central action of diazoxide, as do the findings, in our own study and that of Bingham et al. (27), of an effect of diazoxide on tests of psychomotor speed in human subjects. In a related study, Kishore et al. (35) demonstrated in the rodent model that the extrapancreatic action of diazoxide to suppress hepatic glucose production could be reversed through intracerebroventricular delivery of the K_{ATP} channel blocker glibenclamide. Moreover, they were able to detect diazoxide in the cerebrospinal fluid of rodents after oral ingestion reaching levels of 0.26 ± 0.06 μg/mL 1 h after gavage and 0.78 ± 0.03 μg/mL by 4 h, providing convincing evidence that diazoxide penetrates the blood-brain barrier (BBB) (35). Species differences may affect BBB permeability to diazoxide; however, diazoxide contains an ionizable sulfonyl group that makes it extremely lipid soluble and therefore able to partition into the lipid bilayer for penetration through the BBB (36). These studies support the hypothesis that diazoxide acts to amplify the CRR to acute hypoglycemia through a direct action in the brain.

Our findings contrast with those of Bingham et al. (27) and Raju and Cryer (37), who failed to see significant effects of oral diazoxide on CRRs to hypoglycemia in subjects without diabetes. However, Bingham et al. (27) did report that hypoglycemia-induced peak epinephrine levels were higher after diazoxide (adrenaline 7.37 ± 1.89 vs. 6.18 ± 2.28 nmol/L, respectively; $P = 0.055$). Similarly,

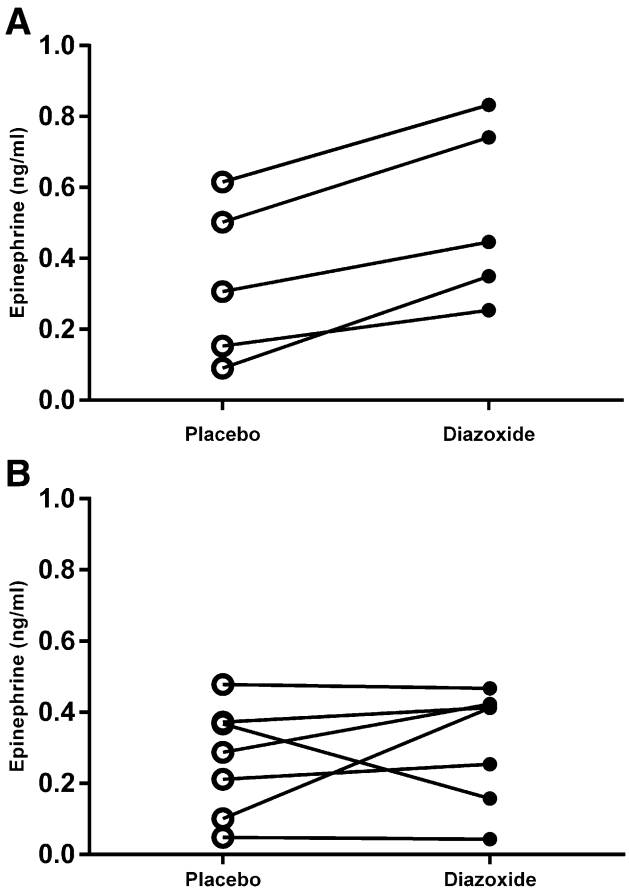

Figure 3—E23K polymorphism in Kir6.2 predicts response to diazoxide during acute hypoglycemia. The magnitude of the epinephrine response during acute hypoglycemia (2.5 mmol/L) is shown after placebo or diazoxide in individuals who expressed wild-type (EE) (*A*) or homo- or heterozygous (KK, EK) (*B*) for this E23K polymorphism for the Kir6.2 channels. Results for each individual under the two conditions are shown.

although Raju and Cryer (37) did not provide actual values to compare epinephrine and norepinephrine responses during the latter stages of the mild hypoglycemic challenge (3.0 mmol/L), these appear greater in those subjects given diazoxide. Both studies used lower doses of diazoxide (5 and 6 mg/kg, respectively), which may have contributed to their failure to see a significant effect. In addition, it is possible that the K_{ATP} channel opening in hypothalamic glucose-sensing neurons during moderate hypoglycemia in individuals without diabetes may already be near maximal. By comparison, subjects with T1D and IAH have impaired glucose sensing, and the K_{ATP} channel is, therefore, less likely to be in the open state and more likely to respond to K_{ATP} channel activators.

In our study, oral diazoxide was able to augment the CRR sufficiently to significantly reduce requirements for exogenous glucose during the clamp procedure, suggesting a real effect on whole-body responses. Despite this, we did not see a statistically significant change in glucose thresholds for counterregulation or for overall symptom responses. However, the subjects in this study had diabetes of relatively long duration, and despite only 5 of the 12 participants having IAH as defined by Gold criteria, 11 of 12 participants had an autonomic symptom threshold of $\leq$3 mmol/L during the clamp studies. Thus, the subjects all had profound defects in symptom and hormonal CRRs to hypoglycemia. It is likely that our study would only have detected large effect sizes in these secondary outcomes. In addition, a limitation of our study is that we calculated thresholds based on the euglycemic period before the induction of hypoglycemia, as reported by others (38). An additional euglycemic control arm to the study would have reduced baseline variability and controlled for effects of time-dependent changes.

An interesting further finding in the current study was the effect of diazoxide on the DSS task. This psychomotor task is often used in hypoglycemia studies and provides a robust and sensitive measure of cognition during hypoglycemia (39). Bingham et al. (27) reported that subjects without diabetes showed a significant prolongation on the 4CRT task during hypoglycemia after diazoxide, but no effect was seen on Stroop and finger-tapping tasks. In the current study, we did not see a significant effect on 4CRT; however, the findings of Bingham et al. (27) are convincing, in that diazoxide and glibenclamide had the opposite effect on 4CRT performance during hypoglycemia. Therefore, drugs that affect the K_{ATP} channel may have widespread effects on brain function, and at least under hypoglycemia conditions, K_{ATP} channel openers lead to an overall reversal of hypoglycemia-induced adaptations in brain glucose sensing and psychomotor performance.

In this study, we also made the interesting observation that the presence of the E23K polymorphism predicted to a large extent whether an individual would respond to diazoxide during hypoglycemia. The exact prevalence of the E23K polymorphism has not been studied in the T1D population. Single nucleotide polymorphisms at codon 23 (E23K,rs5219) in Kir6.2, which is encoded by the *KCNJ11* gene, are associated with type 2 diabetes (40) and also with better response to sulfonylureas (31). The K23 variant of the K_{ATP} channel results in a 60% increase in the likelihood of the K_{ATP} channel being open in the resting phase compared with the wild-type E23 form, and although this variant is in the pore-forming Kir6.2 channel, it demonstrates strong allelic association with a coding variant (A1369S) in the neighboring SUR1 gene, thus predicting response to sulfonylureas (41,42). In our small cohort of 12 participants with well-established T1D, we found 58% carried the K23 variant. This is comparable with the prevalence of 51% (41% hetero- and 10% homozygote) for the E23K polymorphism reported in participants with prediabetes in the Diabetes Prevention Program (41) and with 63% and 59%, respectively, of subjects with type 2 diabetes in the UK Prospective Diabetes Study (UKPDS) and normoglycemic control subjects (43). Although the small size of our study cohort limits the conclusions we can reliably draw from this

analysis, our data suggest that the E23K polymorphism may identify individuals requiring a greater dose of diazoxide to amplify the CRR to hypoglycemia, allowing for a more stratified approach to intervention in the future. Further studies will be required to address this hypothesis.

In summary, we have shown for the first time in human subjects that the K_{ATP} channels are integral to hypoglycemia detection and in the generation of an adequate CRR to acute hypoglycemia. We report that the K_{ATP} channel opener diazoxide, when given orally before a hypoglycemic stimulus to subjects with long-standing T1D and IAH, results in a 37–44% increase in the magnitude of the catecholaminergic counterregulatory hormonal response. Moreover, our data suggest more widespread central actions of diazoxide on neuronal populations involved in psychomotor responses and symptom generation. Finally, we have made the novel observation that the E23K polymorphism in the Kir6.2 subunit of the K_{ATP} channel predicts response to diazoxide therapy during hypoglycemia in T1D. Taken together, we believe clinical trials of longer-term diazoxide therapy in T1D subjects with IAH recruited by genotype are warranted to explore the potential utility of this novel approach to improve hypoglycemia awareness and reduce frequency of severe hypoglycemia.

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References

1. The Diabetes Control and Complications Trial Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes 1997;46:271–286
2. McCrimmon RJ, Sherwin RS. Hypoglycemia in type 1 diabetes. Diabetes 2010;59:2333–2339
3. White NH, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV. Identification of type I diabetic patients at increased risk for hypoglycemia during intensive therapy. N Engl J Med 1983;308:485–491
4. Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. Diabetes 1991;40:223–226
5. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. Diabetes 1988;37:901–907
6. Powell AM, Sherwin RS, Shulman GI. Impaired hormonal responses to hypoglycemia in spontaneously diabetic and recurrently hypoglycemic rats. Reversibility and stimulus specificity of the deficits. J Clin Invest 1993;92:2667–2674
7. Fanelli CG, Epifano L, Rambotti AM, et al. Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. Diabetes 1993;42:1683–1689
8. Little SA, Leelarathna L, Barendse SM, et al. Severe hypoglycaemia in type 1 diabetes mellitus: underlying drivers and potential strategies for successful prevention. Diabetes Metab Res Rev 2014;30:175–190
9. Loveman E, Cave C, Green C, Royle P, Dunn N, Waugh N. The clinical and cost-effectiveness of patient education models for diabetes: a systematic review and economic evaluation. Health Technol Assess 2003;7:iii, 1–190
10. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Effectiveness of continuous glucose monitoring in a clinical care environment: evidence from the Juvenile Diabetes Research Foundation continuous glucose monitoring (JDRF-CGM) trial. Diabetes Care 2010;33:17–22
11. Ly TT, Nicholas JA, Retterath A, Lim EM, Davis EA, Jones TW. Effect of sensor-augmented insulin pump therapy and automated insulin suspension vs standard insulin pump therapy on hypoglycemia in patients with type 1 diabetes: a randomized clinical trial. JAMA 2013;310:1240–1247
12. UK Hypoglycaemia Study Group. Risk of hypoglycaemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. Diabetologia 2007; 50:1140–1147
13. Weinstock RS, Xing D, Maahs DM, et al.; T1D Exchange Clinic Network. Severe hypoglycemia and diabetic ketoacidosis in adults with type 1 diabetes: results from the T1D Exchange clinic registry. J Clin Endocrinol Metab 2013;98:3411–3419
14. Ashford ML, Boden PR, Treherne JM. Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K+ channels. Pflugers Arch 1990;415:479–483
15. Miki T, Liss B, Minami K, et al. ATP-sensitive K+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. Nat Neurosci 2001;4:507–512
16. McCrimmon RJ, Evans ML, Fan X, et al.. Activation of ATP-sensitive potassium channels in the ventromedial hypothalamus amplifies counterregulatory responses to acute hypoglycemia in normal and recurrently hypoglycemic rats. Diabetes 2005;54:3169–3174
17. Evans ML, McCrimmon RJ, Flanagan DE, et al. Hypothalamic ATP-sensitive K + channels play a key role in sensing hypoglycemia and triggering counterregulatory epinephrine and glucagon responses. Diabetes 2004;53:2542–2551
18. Fan X, Ding Y, Cheng H, Gram DX, Sherwin RS, McCrimmon RJ. Amplified hormonal counterregulatory responses to hypoglycemia in rats after systemic delivery of a SUR-1-selective K(+) channel opener? Diabetes 2008;57:3327–3334
19. Liu D, Moberg E, Kollind M, Lins PE, Adamson U, Macdonald IA. Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. Diabetologia 1992;35:287–290
20. Pearson RM. Pharmacokinetics and response to diazoxide in renal failure. Clin Pharmacokinet 1977;2:198–204
21. Deary IJ, Hepburn DA, MacLeod KM, Frier BM. Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. Diabetologia 1993;36:771–777
22. Kortte KB, Horner MD, Windham WK. The trail making test, part B: cognitive flexibility or ability to maintain set? Appl Neuropsychol 2002;9:106–109
23. *Wechsler Memory Scale*. 3rd ed. New York, The Psychological Corporation, 1997
24. Wechsler D. *Manual for the Wechsler Adult Intelligence Scale Revised*. New York, The Psychological Corporation, 1981
25. Deary IJ, Liewald D, Nissan J. A free, easy-to-use, computer-based simple and four-choice reaction time programme: the Deary-Liewald reaction time task. Behav Res Methods 2011;43:258–268

26. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA. Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes. Lancet 1994;344:283–287
27. Bingham E, Hopkins D, Pernet A, Reid H, Macdonald IA, Amiel SA. The effects of KATP channel modulators on counterregulatory responses and cognitive function during acute controlled hypoglycaemia in healthy men: a pilot study. Diabet Med 2003;20:231–237
28. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. Diabetes Care 1994;17:697–703
29. Schwanstecher C, Meyer U, Schwanstecher M. K(IR)6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic beta-cell ATP-sensitive K(+) channels. Diabetes 2002;51:875–879
30. Hamming KS, Soliman D, Matemisz LC, et al. Coexpression of the type 2 diabetes susceptibility gene variants KCNJ11 E23K and ABCC8 S1369A alter the ATP and sulfonylurea sensitivities of the ATP-sensitive K(+) channel. Diabetes 2009;58:2419–2424
31. Javorsky M, Klimcakova L, Schroner Z, et al. KCNJ11 gene E23K variant and therapeutic response to sulfonylureas. Eur J Intern Med 2012;23:245–249
32. Mayer J. Glucostatic mechanism of regulation of food intake. N Engl J Med 1953;249:13–16
33. Beall C, Hamilton DL, Gallagher J, et al. Mouse hypothalamic GT1-7 cells demonstrate AMPK-dependent intrinsic glucose-sensing behaviour. Diabetologia 2012;55:2432–2444
34. Saberi M, Bohland M, Donovan CM. The locus for hypoglycemic detection shifts with the rate of fall in glycemia: the role of portal-superior mesenteric vein glucose sensing. Diabetes 2008;57:1380–1386
35. 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
36. Kamp F, Kizilbash N, Corkey BE, Berggren PO, Hamilton JA. Sulfonylureas rapidly cross phospholipid bilayer membranes by a free-diffusion mechanism. Diabetes 2003;52:2526–2531
37. Raju B, Cryer PE. Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraislet insulin hypothesis in humans. Diabetes 2005;54:757–764
38. Leelarathna L, Little SA, Walkinshaw E, et al. Restoration of self-awareness of hypoglycemia in adults with long-standing type 1 diabetes: hyperinsulinemic-hypoglycemic clamp substudy results from the HypoCOMPaSS trial. Diabetes Care 2013;36:4063–4070
39. Geddes J, Schopman JE, Zammitt NN, Frier BM. Prevalence of impaired awareness of hypoglycaemia in adults with type 1 diabetes. Diabet Med 2008;25:501–504
40. Hani EH, Boutin P, Durand E, et al. Missense mutations in the pancreatic islet beta cell inwardly rectifying K+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of type II diabetes mellitus in Caucasians. Diabetologia 1998;41:1511–1515
41. Florez JC, Burtt N, de Bakker PI, et al. Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. Diabetes 2004;53:1360–1368
42. Sesti G, Laratta E, Cardellini M, et al. The E23K variant of KCNJ11 encoding the pancreatic beta-cell adenosine 5′-triphosphate-sensitive potassium channel subunit Kir6.2 is associated with an increased risk of secondary failure to sulfonylurea in patients with type 2 diabetes. J Clin Endocrinol Metab 2006;91:2334–2339
43. Gloyn AL, Hashim Y, Ashcroft SJ, Ashfield R, Wiltshire S, Turner RC; UK Prospective Diabetes Study (UKPDS 53). Association studies of variants in promoter and coding regions of beta-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with Type 2 diabetes mellitus (UKPDS 53). Diabet Med 2001;18:206–212