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

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OBJECTIVE—In the pancreatic β-cell, ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channels couple metabolism with excitability and consist of Kir6.2 and SUR1 subunits encoded by KCNJ11 and ABCC8, respectively. Sulfonylureas, which inhibit the K\(_{\text{ATP}}\) channel, are used to treat type 2 diabetes. Rare activating mutations cause neonatal diabetes, whereas the common variants, E23K in KCNJ11 and S1369A in ABCC8, are in strong linkage disequilibrium, constituting a haplotype that predisposes to type 2 diabetes. To date it has not been possible to establish which of these represents the etiological variant, and functional studies are inconsistent. Furthermore, there have been no studies of the S1369A variant or the combined effect of the two on K\(_{\text{ATP}}\) channel function.

RESEARCH DESIGN AND METHODS—The patch-clamp technique was used to study the nucleotide sensitivity and sulfonylurea inhibition of recombinant human K\(_{\text{ATP}}\) channels containing either the E23K/A1369 or E23K/S1369A variants.

RESULTS—ATP sensitivity of the K\(_{\text{ATP}}\) channel was decreased in the E23K/A1369 variant (half-maximal inhibitory concentration [IC\(_{50}\)] = 8.0 vs. 2.5 μmol/l for the E23K/S1369A variant), although there was no difference in ADP sensitivity. The E23K/A1369 variant also displayed increased inhibition by gliclazide, an A-site sulfonylurea drug (IC\(_{50}\) = 52.7 vs. 188.7 nmol/l for the E23K/S1369A variant), but not by glibenclamide (AB site) or repaglinide (B site).

CONCLUSIONS—Our findings indicate that the common E23K/A1369 variant K\(_{\text{ATP}}\) channel displays decreased ATP inhibition that may contribute to the observed increased risk for type 2 diabetes. Moreover, the increased sensitivity of the E23K/A1369 variant to the A-site sulfonylurea drug gliclazide may provide a pharmacogenomic therapeutic approach for patients with type 2 diabetes who are homozygous for both risk alleles. Diabetes 58: 2419–2424, 2009

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RESEARCH DESIGN AND METHODS

Molecular biology. The human K\(_{\text{ATP}}\) channel Kir6.2 and SUR1 subunit clones were kindly provided by J. Bryan (Pacific Northwest Diabetes Research Institute, Seattle, WA). The E23K and S1369A variants were introduced into the KCNJ11 and ABCC8 cDNAs, respectively, using site-directed mutagenesis (QuikChange; Stratagene).

Cell culture, transfection, and electrophysiology. Cultured tsA201 cells were transfected with the KCNJ11 and ABCC8 clones using the calcium phosphate precipitation technique (13). Transfected cells were identified...
using fluorescent optics in combination with coexpression of a green fluorescent protein plasmid (Life Technologies, Gaithersburg, MD). Macroscopic K\textsubscript{ATP} channel recordings were then performed 48–72 h after transfection. The inside-out patch-clamp technique was used to measure macroscopic K\textsubscript{ATP} channel currents in transfected tsA201 cells as described in detail previously (13).

**Experimental compounds.** MgATP and MgADP (Sigma, Oakville, Ontario) were prepared as 10 mmol/l stocks in ddH\textsubscript{2}O immediately prior to use. Glibenclamide, gliclazide, and repaglinide (Sigma, Oakville, Ontario) were prepared as 10 mmol/l stocks in DMSO and stored at \(-20^\circ\text{C}\). DMSO concentration was maintained at 0.1% in all experimental solutions.

**Statistical analysis.** Macroscopic K\textsubscript{ATP} channel currents were normalized and expressed as changes in current relative to control (i.e., normalized K\textsubscript{ATP} channel current = \(I_{\text{test}}/I_{\text{control}}\)). Single-channel analysis was performed using pClamp v. 10.0 software (Axon Instruments). Statistical significance was assessed using the unpaired Student’s \(t\) test or one-way ANOVA with a Bonferroni post hoc test. \(P < 0.05\) was considered statistically significant. Data are expressed as means \(\pm\) SE.

**RESULTS**

Residue S1369 is proximal to the second nucleotide-binding domain in SUR1, which forms part of the MgATP- and MgADP-sensing region in SUR1 that is a key regulator of K\textsubscript{ATP} channel activity and, hence, insulin secretion (3,14). However, the direct effects of the K23/A1369 variant on human K\textsubscript{ATP} channel nucleotide sensitivities have not been investigated.

Therefore, to gain insights into the nucleotide regulation of K23/A1369 variant K\textsubscript{ATP} channel activity, the MgATP and MgADP sensitivities of recombinant human K\textsubscript{ATP} channels containing either the K23/S1369 or the E23/S1369 variant were compared. Our results indicate that the K23/A1369 variant decreases the MgATP sensitivity of the K\textsubscript{ATP} channel (half-maximal inhibitory concentration
[IC\textsubscript{50}] = 8.0 ± 0.8 vs. 2.5 ± 0.2 μmol/l for the E23/S1369 variant, *P < 0.05; Fig. 1A and B). Extrapolation of the MgATP concentration-inhibition curve to physiological millimolar intracellular MgATP levels (1–5 mmol/l) predicted that the shift in IC\textsubscript{50} may result in the K23/A1369 variant remaining slightly more active compared with the E23/S1369 variant (Fig. 1B, inset). Subsequent single-channel experiments confirmed this prediction with the open probability of the K23/A1369 variant being significantly greater than the E23/S1369 variant at 1 mmol/l MgATP but not at 0 mmol/l MgATP (Fig. 1C–F). To determine whether one or both of the K23 or A1369 variants account for the reduced MgATP sensitivity, MgATP concentration-inhibition curves were constructed from quasi-heterologous K\textsubscript{ATP} channels expressing either E23/A1369 or K23/S1369. These results indicate that it is the ABCC8 A1369 variant, not the KCNJ11 K23 variant, that confers the reduced MgATP sensitivity to the K\textsubscript{ATP} channel complex (IC\textsubscript{50} = 8.2 ± 1.6 vs. 3.2 ± 0.3 μmol/l for E23/A1369 vs. K23/S1369, respectively; Fig. 1G).

The intracellular ATP-to-ADP ratio is a major determinant of K\textsubscript{ATP} channel activity because MgADP antagonizes the inhibitory effects of ATP, and rare monogenic mutations in ABCC8 that reduce MgADP antagonism decrease channel activity and cause hyperinsulinism (17). Accordingly, the stimulatory effects of varying concentrations of MgADP were tested in the presence of 0.1 mmol/l MgATP. However, no significant differences were observed between the E23/S1369 and K23/A1369 K\textsubscript{ATP} channel variants (Fig. 2A and B)

The K\textsubscript{ATP} channel is the molecular target for sulfonylurea and glinide drugs that are commonly used to stimulate insulin secretion in type 2 diabetes. Interestingly, recent clinical data suggest that diabetic patients who are homozygous for the A1369 risk allele (A/A) are more responsive to gliclazide therapy (17). However, it is unknown whether this is due to a direct effect on the K\textsubscript{ATP} channel because the inhibitory profile of gliclazide and other drugs on the K23/A1369 variant K\textsubscript{ATP} channel has not been determined.

Sulfonylurea and glinide drugs can be grouped according to their binding to the A, B, or AB sites in the K\textsubscript{ATP} channel complex (3,16,17). The A site is located close to SUR1 transmembrane segments 14–16, and the S1237Y mutation in this region (Fig. 3A) abolishes A-site drug inhibition (18). Two regions of the K\textsubscript{ATP} channel contribute to the B site: the intracellular loop between SUR1 transmembrane segments 5 and 6 and the NH\textsubscript{2}-terminus of Kir6.2 (16) (Fig. 3A). Figure 3F shows the structures of the glinide repaglinide (B site) and the sulfonylureas glibenclamide (AB site) and gliclazide (A site). The SUR1 residue S1369 is in close proximity to the A site (Fig. 3A).

Therefore, the A1369 variant may contribute to altered K\textsubscript{ATP} channel sensitivity to A-site drugs such as gliclazide. Gliclazide (300 nmol/l) inhibited the K23/A1369 variant to a greater extent than the E23/S1369 variant (Fig. 3C and D). Construction of gliclazide concentration-inhibition curves revealed that the K23/A1369 variant was 3.5-fold more sensitive to gliclazide inhibition than the E23/S1369 variant (IC\textsubscript{50} 52.7 ± 11.1 vs. 188.7 ± 32.6 mmol/l, respectively; Fig. 3E). Because the K23/A1369 K\textsubscript{ATP} channel variant may also alter the potency of other drug classes, the effects of glibenclamide (AB site) and repaglinide (B site) were tested. In direct contrast to the observed effects of gliclazide, no significant differences in either glibenclamide (3 nmol/l) or repaglinide (10 nmol/l) inhibition were found between the K23/A1369 and E23/S1369 variant K\textsubscript{ATP} channels (Fig. 3F). It is possible that gliclazide inhibition may be affected by intracellular MgADP. In the presence of 0.1 mmol/l MgATP and 0.1 mmol/l MgADP, 300 nmol/l gliclazide still elicited a significantly greater inhibition of the K23/A1369 K\textsubscript{ATP} channel variant than the E23/S1369 variant (Fig. 4A–C).

The data presented indicate that the K23/A1369 variant K\textsubscript{ATP} channel is more sensitive to inhibition by gliclazide but not glibenclamide or repaglinide. However, the relative individual contributions of the ABCC8 A1369 or KCNJ11 K23 variants to gliclazide sensitivity have not been determined. Therefore, gliclazide inhibition was measured in quasi-heterologous K\textsubscript{ATP} channels containing either the E23/A1369 or K23/S1369 variant combinations. E23/A1369 K\textsubscript{ATP} channels displayed a significantly greater gliclazide inhibition than K23/S1369 K\textsubscript{ATP} channels, which was similar in magnitude to that observed in the increased diabetes risk for the K23/A1369 variant K\textsubscript{ATP} channel (Fig. 4D–F). Results from these experiments indicate that the enhanced gliclazide sensitivity in the K23/A1369 K\textsubscript{ATP}

FIG. 2. A: Representative macroscopic current recordings of the MgADP stimulatory effects of 0.1 mmol/l MgADP in the presence of 0.1 mmol/l MgATP. B: Concentration response curves for the stimulatory effects of increasing MgADP concentrations in the presence of 0.1 mmol/l MgATP. Results show no significant differences in MgADP stimulation between the E23/S1369 and K23/A1369 haplotypes across a range of MgADP concentrations (P > 0.05). n = 3–10 patches per group.
channel variant is conferred by the ABCC8 A1369 variant and not the KCNJ11 K23 variant.

**DISCUSSION**

Previous studies have investigated the properties of K\textsubscript{ATP} channels containing the KCNJ11 K23 variant (9–11), although >95% of people with two copies of K23 are also homozygous for A1369 (12). Therefore, this study is the first to document the properties and pharmacology of the most commonly found K\textsubscript{ATP} channel variant that contains both K23 and A1369 risk alleles. Our study reveals novel differences in both the MgATP and sulfonylurea sensitivity of this variant K\textsubscript{ATP} channel.

With respect to MgATP sensitivity, the moderate rightward shift in IC\textsubscript{50} for MgATP inhibition seen in the K23/A1369 variant results in increased basal K\textsubscript{ATP} channel activity at physiological MgATP levels. In direct contrast to the rare monogenic K\textsubscript{ATP} channel mutations that cause neonatal diabetes and drastically decreased MgATP inhibition, a modest increase in K23/A1369 variant K\textsubscript{ATP} channel activity may predispose to type 2 diabetes in combination with other factors. Indeed, we have previously shown that the K23 variant increases the sensitivity of the K\textsubscript{ATP} channel to activation by intracellular acyl CoAs (11,13). K\textsubscript{ATP} channels encoded by the KCNJ11 and ABCC8 genes are also expressed in pancreatic α-cells and hypothalamic neurons that centrally regulate glucose/energy homeostasis (19). Therefore, it is plausible that subtle increases in the activity of K23/A1369 variant K\textsubscript{ATP} channels may alter glucagon secretion and centrally mediated glucose homeostasis, further contributing to the development of type 2 diabetes.

The molecular mechanism for the reduced ATP inhibition observed in K\textsubscript{ATP} channels expressing the K23/A1369 variant proteins is of importance. Free ATP inhibits K\textsubscript{ATP} channel activity via binding to the Kir6.2 subunit, whereas, paradoxically, MgATP can activate the channel via intrinsic MgATPase activity of the nucleotide-binding folds in SUR1, resulting in production of MgADP that may stimulate channel activity (2). In direct contrast to a previous study on the KCNJ11 K23 variant (20), our results indicate that the stimulatory effects of MgADP are unaltered in the K23/A1369 variant K\textsubscript{ATP} channel, suggesting that the molecular mechanism for decreased ATP inhibition does not involve altered MgADP sensitivity per se. Our results also show that the observed decrease in ATP inhibition in the
K23/A1369 variant KATP channel results from a direct effect of the ABCC8 A1369 risk allele reducing ATP inhibition (9), perhaps via mild increases in the intrinsic KATP channel MgATPase activity. Indeed, several rare heterozygous mutations in ABCC8 that cause neonatal diabetes (R1380L and R1380C) act by increasing MgATPase activity (21). Interestingly, the location of the ABCC8 S1369 residue is in close proximity to the MgATPase catalytic site and residue R1380 in the SUR1 nucleotide-binding fold 2 (22).

Sulfonylurea and glinide drugs that inhibit KATP channels are in extensive clinical use to stimulate insulin secretion in patients with type 2 diabetes (3). Glibenclamide is an AB-site ligand and is the most widely used sulfonylurea, whereas gliclazide is an A-site ligand selectively inhibiting KATP channels containing the SUR1 isoform, potentially mitigating any cardiotoxicity that has been associated with glibenclamide monotherapy (23,24). Our results indicate that the K23/A1369 variant KATP channel is 3.5-fold more sensitive to gliclazide. These findings are the first to directly demonstrate altered sulfonylurea sensitivities of the K23/A1369 variant KATP channel and identify the ABCC8 A1369 risk allele as conferring this effect upon the K23/A1369 variant KATP channel. These results provide a molecular mechanism for the increase in clinical efficacy of gliclazide in subjects with type 2 diabetes who are homozygous for the A1369 allele variant (15).

In conclusion, this study provides the first evidence that the ABCC8 S1369A variant alters the properties of the KATP channel that may contribute to the increased risk for type 2 diabetes associated with the K23/A1369 risk haplotype. The increased gliclazide sensitivity observed in the K23/A1369 variant KATP channel (afforded by the ABCC8 A1369 risk allele) encourages the study of sulfonylurea pharmacogenomics in larger cohorts and supports a rationale for tailoring pharmacotherapy in the ~20% of type 2 diabetic patients who carry two copies of these risk alleles.

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REFERENCES

1. Prokopenko I, McCarthy MI, Lindgren CM. Type 2 diabetes: new genes, new understanding. Trends Genet 2008;24:613–621

2. Bryan J, Munoz A, Zhang X, Dufer M, Drews G, Krippeit-Drews P, Aguilar-Bryan L. ABCB8 and ABCC9: ABC transporters that regulate K+ channels. Pflugers Arch 2007;453:703–718

3. Bryan J, Crane A, Vila-Carriles WH, Babenko AP, Aguilar-Bryan L. Insulin secretagogues, sulfonylurea receptors and KATP channels. Curr Pharm Des 2005;11:2099–2176

4. Babenko AP, Polak M, Cvej B, Huisja K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Proguel P. Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. N Engl J Med 2006;355:456–466

5. Gloy AL, Pearson ER, Antcliff JP, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Holmes J, Edghill EL, Freysing TM, Temple IK, Mackay DJ, Shield JP, Sumnik Z, van Rijn A, Wales JK, Clark P, Gorman S, Aisenberg J, Eillard S, Njolstad PR, Ashcroft FM, Hattersley AT. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 2004;350:1838–1849

6. Gloy AL, Reimann F, Girard C, Edghill EL, Proks P, Pearson ER, Temple IK, Mackay DJ, Shield JP, Freedemberg D, Noyes K, Eillard S, Ashcroft FM, Gribble FM, Hattersley AT. Relapsing diabetes can result from moderately activating mutations in KCNJ11. Hum Mol Genet 2005;14:925–934

7. Gloy AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Freysing TM. Large-scale association studies of variants in genes encoding the pancreatic β-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes 2003;52:568–572

8. Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glumer C, Thorsteinsson B, Borch-Johnsen K, Hansen T, Pedersen O. The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. Diabetes 2003;52:573–577

9. Schwanstecher C, Meyer U, Schwanstecher M. Kir6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β-cell ATP-sensitive K+ channels. Diabetes 2002;51:875–879

10. Sakura H, Wat N, Horton V, Mills H, Turner RC, Ashcroft FM. Sequence variations in the human Kir6.2 gene, a subunit of the β-cell ATP-sensitive K-channel: no association with NIDDM in white Caucasian subjects or evidence of abnormal function when expressed in vitro. Diabetologia 1996;39:1238–1236

11. Riedel MJ, Boora P, Steckley D, de Vries G, Light PE. Kir6.2 polymorphisms sensitize β-cell ATP-sensitive potassium channels to activation by acyl CoAs: a possible cellular mechanism for increased susceptibility to type 2 diabetes? Diabetes 2003;52:2630–2635

12. Florez JC, Burtt N, de Bakker PI, Almgren P, Tuomi T, Holmquist J, Gaudet D, Hudson TJ, Schaffner SF, Duly MJ, Hirschhorn JN, Groop L, Abshuler D. Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. Diabetes 2004;53:1360–1368

13. Riedel MJ, Light PE. Saturated and cis/trans unsaturated acyl CoA esters differentially regulate wild-type and polymorphic β-cell ATP-sensitive K+ channels. Diabetes 2005;54:2070–2079

14. Nichols CG, Shyn SL, Nestorowicz A, Glaser B, Clement JP, Gonzalez G, Aguilar-Bryan L, Permutt MA, Bryan J. Adenosine diphosphate as an intracellular regulator of insulin secretion. Science 1996;272:1785–1787

15. Peng Y, Mao G, Ren X, Xing H, Tang G, Li Q, Li X, Sun L, Yang J, Ma W, Wang X, Xu X, Ser1369Ala variant in sulfonylurea receptor gene ABCC8 is associated with antidiabetic efficacy of gliclazide in Chinese type 2 diabetic patients. Diabetes Care 2008;31:1939–1944

16. Vila-Carriles WH, Zhao G, Bryan J. Defining a binding pocket for sulfonylureas in ATP-sensitive potassium channels. Faseb J 2007;21:18–25

17. Winkler M, Stephan D, Bieger S, Kuhner P, Wolf F, Quast U. Testing the bipartite model of the sulfonylurea receptor binding site: binding of A-, B-, and A + B-site ligands. J Pharmacol Exp Ther 2007;322:701–708

18. Ashfield R, Gribble FM, Ashcroft SJ, Ashcroft FM. Identification of the high-affinity tolbutamide site on the SUR1 subunit of the KATP channel. Diabetes 1999;48:1341–1347

19. Minami K, Miki T, Kadowaki T, Seino S. Roles of ATP-sensitive K+ channels as metabolic sensors: studies of Kir6.x null mice. Diabetes 2004;53(Suppl. 3):S176–S180

20. Schwanstecher C, Neugebauer B, Schulz M, Schwanstecher M. The common single nucleotide polymorphism E23K in Kir6.2 sensitizes pancreatic β-cell ATP-sensitive potassium channels toward activation through nucleoside diphosphates. Diabetes 2002;51(Suppl. 3):S363–S367

21. de Wet H, Rees MG, Shimomura K, Aittoniemi J, Patch AM, Flanagan SE, Eillard S, Hattersley AT, Sansom MS, Ashcroft FM. Increased ATPase activity produced by mutations at arginine-1380 in nucleotide-binding domain 2 of ABCC8 causes neonatal diabetes. Proc Natl Acad Sci U S A 2007;104:18988–18992

22. Masia R, Nichols CG. Functional clustering of mutations in the dimer interface of the nucleotide binding folds of the sulfonylurea receptor. J Biol Chem 2008;283:30322–30329

23. McAlistler FA, Urich DT, Majumdar SR, Johnson JA. The risk of heart failure in patients with type 2 diabetes treated with oral agent monotherapy. Eur J Heart Fail 2008;10:703–708

24. Evans JM, Ogston SA, Emslie-Smith A, Morris AD. Risk of mortality and adverse cardiovascular outcomes in type 2 diabetes: a comparison of patients treated with sulfonylureas and metformin. Diabetesologia 2006;49:930–936