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$_{ATP}$) channels couple metabolism with excitability and consist of Kir6.2 and SUR1 subunits encoded by *KCNJ11* and *ABCC8*, respectively. Sulfonylureas, which inhibit the K$_{ATP}$ 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$_{ATP}$ channel function.

RESEARCH DESIGN AND METHODS—The patch-clamp technique was used to study the nucleotide sensitivity and sulfonylurea inhibition of recombinant human K$_{ATP}$ channels containing either the K23/A1369 or E23/S1369 variants.

RESULTS—ATP sensitivity of the K$_{ATP}$ channel was decreased in the K23/A1369 variant (half-maximal inhibitory concentration [IC$_{50}$] = 8.0 vs. 2.5 μmol/l for the E23/S1369 variant), although there was no difference in ADP sensitivity. The K23/A1369 variant also displayed increased inhibition by gliclazide, an A-site sulfonylurea drug (IC$_{50}$ = 52.7 vs. 188.7 nmol/l for the E23/S1369 variant), but not by glibenclamide (AB site) or repaglinide (B site).

CONCLUSIONS—Our findings indicate that the common K23/A1369 variant K$_{ATP}$ channel displays decreased ATP inhibition that may contribute to the observed increased risk for type 2 diabetes. Moreover, the increased sensitivity of the K23/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**

Recent large-scale human genetic studies have made dramatic progress in identifying type 2 diabetes susceptibility genes, increasing the list from three genes (*PPARG*, *KCNJ11*, and *TCF7L2*) to nearly 20 genes in the last 2 years (1). Despite this rapid progress, what the precise causal variant is and how the variant increases susceptibility to type 2 diabetes is still unknown in the majority of cases. Even the widely accepted type 2 diabetes susceptibility gene *KCNJ11* has not yet had the mutational mechanisms fully elucidated.

In pancreatic β-cells and the central nervous system, ATP-sensitive K$^+$ (K$_{ATP}$) channels are composed of the Kir6.2 and SUR1 subunits encoded by the *KCNJ11* and *ABCC8* genes, respectively. K$_{ATP}$ channels act as key transducers of metabolic signals to excitability in many cell types including the regulation of insulin secretion (2), and the K$_{ATP}$ channel is the target for commonly used antidiabetic sulfonylurea drugs (3). The importance of the K$_{ATP}$ channel in diabetes is highlighted by the fact that rare heterozygous activating mutations in *KCNJ11* or *ABCC8* cause diabetes with varying clinical severities (4–6).

One of the first reproducibly associated type 2 diabetes susceptibility signals identified was the common E23K (rs5219) variant of *KCNJ11* (7,8). Functional studies were subsequently performed, but the results were inconsistent (9–11). Moreover, fine mapping in the region demonstrated the difficulty in identifying the causal variant when a second nonsynonymous (S1369A; rs757110) variant in the neighboring *ABCC8* gene was shown to be in complete linkage disequilibrium with the E23K *KCNJ11* variant (12). The implications of this were that *1*) it was not possible from the genetic evidence to say which variant is actually the etiological variant and *2*) individuals who carried the K risk allele of the E23K variant also carried the A risk allele of the S1369A variant. Consequently, functional studies to investigate the mutational mechanism need to include both variants.

RESEARCH DESIGN AND METHODS

Molecular biology. The human K$_{ATP}$ 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
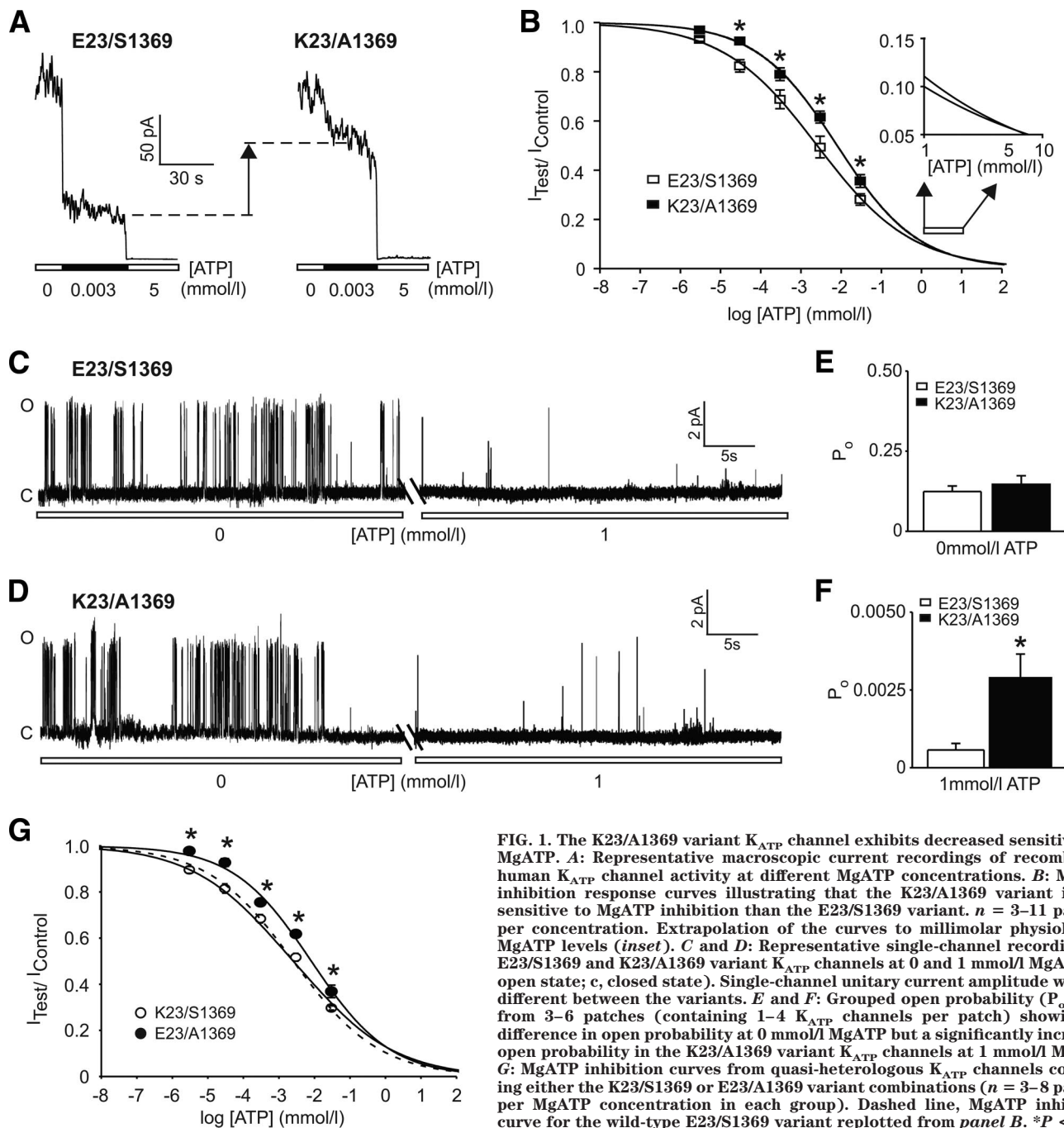
FIG. 1. The K23/A1369 variant K$_{ATP}$ channel exhibits decreased sensitivity to MgATP. *A*: Representative macroscopic current recordings of recombinant human K$_{ATP}$ channel activity at different MgATP concentrations. *B*: MgATP inhibition response curves illustrating that the K23/A1369 variant is less sensitive to MgATP inhibition than the E23/S1369 variant. n = 3–11 patches per concentration. Extrapolation of the curves to millimolar physiological MgATP levels (*inset*). *C* and *D*: Representative single-channel recordings of E23/S1369 and K23/A1369 variant K$_{ATP}$, channels at 0 and 1 mmol/l MgATP (o, open state; c, closed state). Single-channel unitary current amplitude was not different between the variants. *E* and *F*: Grouped open probability (P$_o$) data from 3–6 patches (containing 1–4 K$_{ATP}$ channels per patch) showing no difference in open probability at 0 mmol/l MgATP but a significantly increased open probability in the K23/A1369 variant K$_{ATP}$ channels at 1 mmol/l MgATP. *G*: MgATP inhibition curves from quasi-heterologous K$_{ATP}$ channels containing either the K23/S1369 or E23/A1369 variant combinations (n = 3–8 patches per MgATP concentration in each group). Dashed line, MgATP inhibition curve for the wild-type E23/S1369 variant replotted from *panel B*. *$P < 0.05$.

using fluorescent optics in combination with coexpression of a green fluorescent protein plasmid (Life Technologies, Gaithersburg, MD). Macroscopic K$_{ATP}$ channel recordings were then performed 48–72 h after transfection. The inside-out patch-clamp technique was used to measure macroscopic K$_{ATP}$ 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$_2$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°C. DMSO concentration was maintained at 0.1% in all experimental solutions.

Statistical analysis. Macroscopic K$_{ATP}$ channel currents were normalized and expressed as changes in current relative to control (i.e., normalized K$_{ATP}$ channel current = I$_{test}$/I$_{control}$). 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 Bonnferoni post hoc test. $P < 0.05$ was considered statistically significant. Data are expressed as means ± 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$_{ATP}$ channel activity and, hence, insulin secretion (3,14). However, the direct effects of the K23/A1369 variant on human K$_{ATP}$ channel nucleotide sensitivities have not been investigated.

Therefore, to gain insights into the nucleotide regulation of K23/A1369 variant K$_{ATP}$ channel activity, the MgATP and MgADP sensitivities of recombinant human K$_{ATP}$ channels containing either the K23/A1369 or the E23/S1369 variants were compared. Our results indicate that the K23/A1369 variant decreases the MgATP sensitivity of the K$_{ATP}$ channel (half-maximal inhibitory concentration
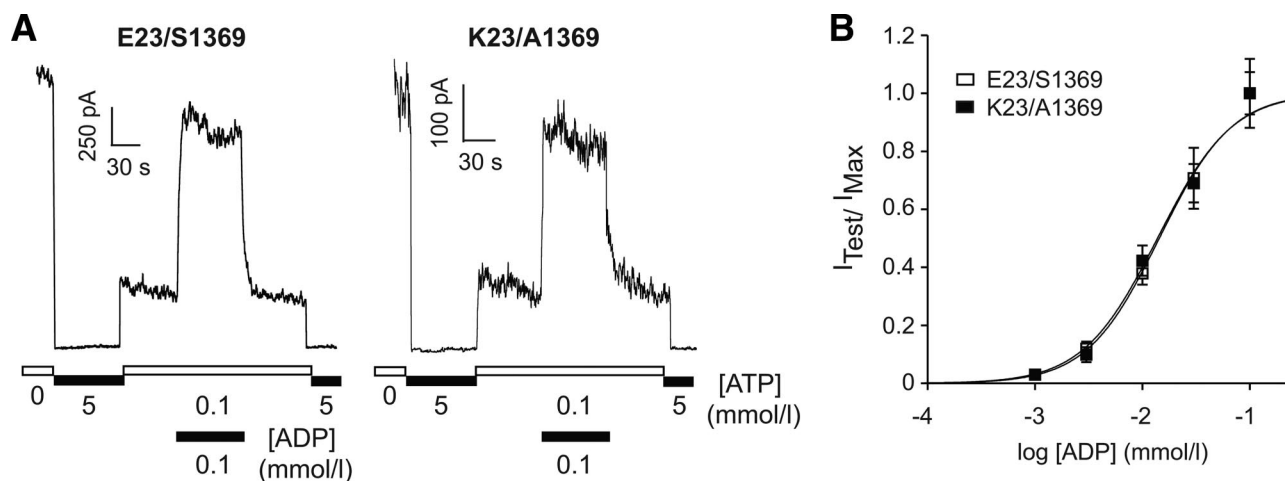
FIG. 2. *A*: Representative macroscopic current recordings of the MgADP stimulatory effects of 0.1 mmol/l MgADP in the presence of 0.1 mmol/l MgATP. *B*: Concentration response curves for the stimulatory effects of increasing MgADP concentrations in the presence of 0.1 mmol/l MgATP. Results show no significant 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.

[IC$_{50}$] = 8.0 ± 0.8 vs. 2.5 ± 0.2 μmol/l for the E23/S1369 variant, $P < 0.05$; Fig. 1*A* 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$_{50}$ may result in the K23/A1369 variant remaining slightly more active compared with the E23/S1369 variant (Fig. 1*B*, *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. 1*C–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$_{ATP}$ 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$_{ATP}$ channel complex (IC$_{50}$ = 8.2 ± 1.6 vs. 3.2 ± 0.3 μmol/l for E23/A1369 vs. K23/S1369, respectively; Fig. 1*G*).

The intracellular ATP-to-ADP ratio is a major determinant of K$_{ATP}$ 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 (14). 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$_{ATP}$ channel variants (Fig. 2*A* and *B*).

The K$_{ATP}$ 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 (15). However, it is unknown whether this is due to a direct effect on the K$_{ATP}$ channel because the inhibitory profile of gliclazide and other drugs on the K23/A1369 variant K$_{ATP}$ 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$_{ATP}$ 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. 3*A*) abolishes A-site drug inhibition (18). Two regions of the K$_{ATP}$ channel contribute to the B site: the intracellular loop between SUR1 transmembrane segments 5 and 6 and the NH$_2$-terminus of Kir6.2 (16) (Fig. 3*A*). Figure 3*B* 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. 3*A*). Therefore, the A1369 variant may contribute to altered K$_{ATP}$ 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. 3*C* 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$_{50}$ 52.7 ± 11.1 vs. 188.7 ± 32.6 nmol/l, respectively; Fig. 3*E*). Because the K23/A1369 K$_{ATP}$ 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$_{ATP}$ channels (Fig. 3*F*). 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$_{ATP}$ channel variant than the E23/S1369 variant (Fig. 4*A–C*).

The data presented indicate that the K23/A1369 variant K$_{ATP}$ 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$_{ATP}$ channels containing either the E23/A1369 or K23/S1369 variant combinations. E23/A1369 K$_{ATP}$ channels displayed a significantly greater gliclazide inhibition than K23/S1369 K$_{ATP}$ channels, which was similar in magnitude to that observed in the increased diabetes risk for the K23/A1369 variant K$_{ATP}$ channel (Fig. 4*D–F*). Results from these experiments indicate that the enhanced gliclazide sensitivity in the K23/A1369 K$_{ATP}$
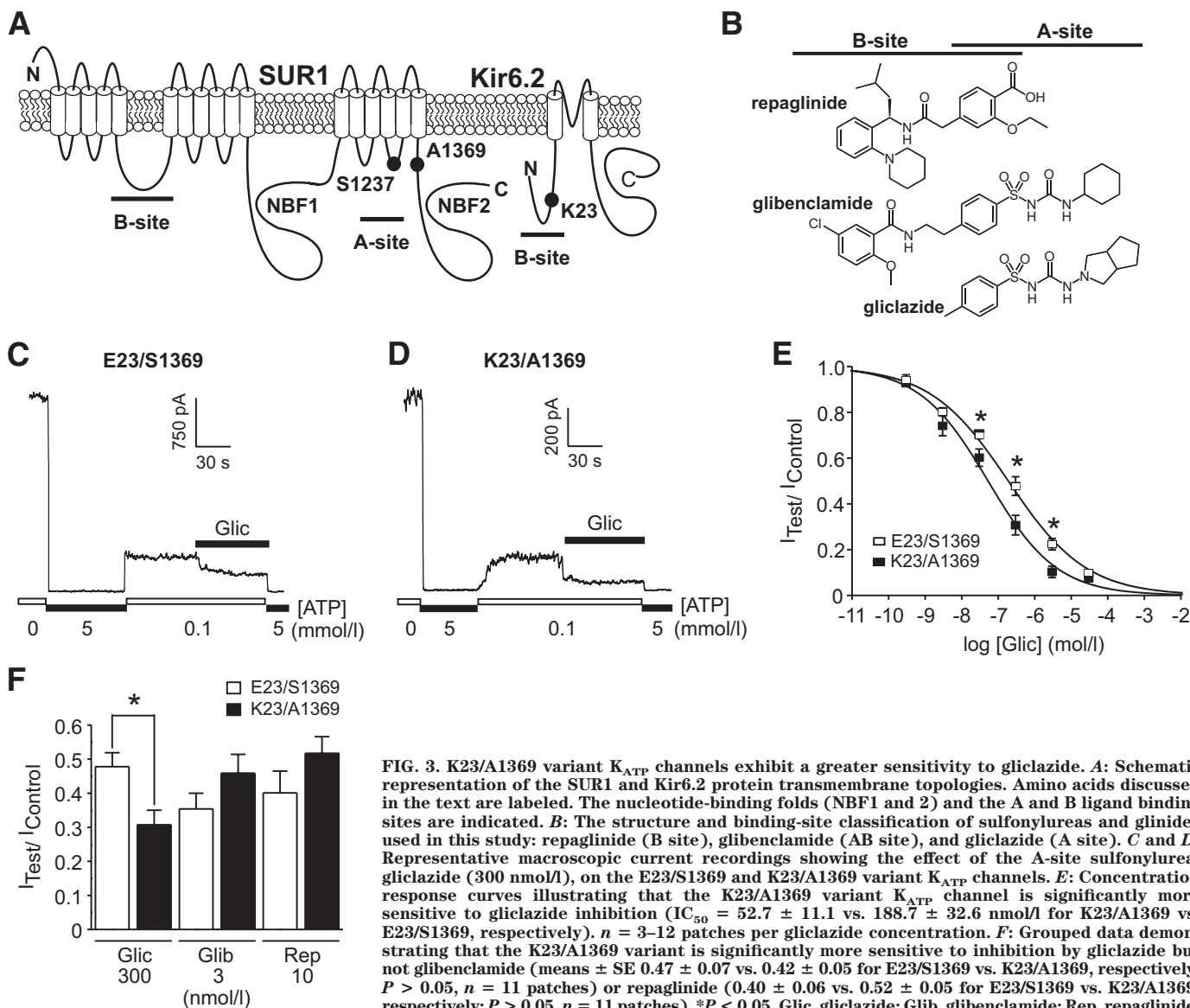
FIG. 3. K23/A1369 variant K$_{ATP}$ channels exhibit a greater sensitivity to gliclazide. *A*: Schematic representation of the SUR1 and Kir6.2 protein transmembrane topologies. Amino acids discussed in the text are labeled. The nucleotide-binding folds (NBF1 and 2) and the A and B ligand binding sites are indicated. *B*: The structure and binding-site classification of sulfonylureas and glinides used in this study: repaglinide (B site), glibenclamide (AB site), and gliclazide (A site). *C* and *D*: Representative macroscopic current recordings showing the effect of the A-site sulfonylurea, gliclazide (300 nmol/l), on the E23/S1369 and K23/A1369 variant K$_{ATP}$ channels. *E*: Concentration response curves illustrating that the K23/A1369 variant K$_{ATP}$ channel is significantly more sensitive to gliclazide inhibition (IC$_{50}$ = 52.7 ± 11.1 vs. 188.7 ± 32.6 nmol/l for K23/A1369 vs. E23/S1369, respectively). *n* = 3–12 patches per gliclazide concentration. *F*: Grouped data demonstrating that the K23/A1369 variant is significantly more sensitive to inhibition by gliclazide but not glibenclamide (means ± SE 0.47 ± 0.07 vs. 0.42 ± 0.05 for E23/S1369 vs. K23/A1369, respectively; *P* > 0.05, *n* = 11 patches) or repaglinide (0.40 ± 0.06 vs. 0.52 ± 0.05 for E23/S1369 vs. K23/A1369, respectively; *P* > 0.05, *n* = 11 patches). *P < 0.05. Glic, gliclazide; Glib, glibenclamide; Rep, repaglinide.

channel variant is conferred by the *ABCC8* A1369 variant and not the *KCNJ11* K23 variant.

DISCUSSION

Previous studies have investigated the properties of K$_{ATP}$ 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$_{ATP}$ 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$_{ATP}$ channel.

With respect to MgATP sensitivity, the moderate rightward shift in IC$_{50}$ for MgATP inhibition seen in the K23/A1369 variant results in increased basal K$_{ATP}$ channel activity at physiological MgATP levels. In direct contrast to the rare monogenic K$_{ATP}$ channel mutations that cause neonatal diabetes and drastically decreased MgATP inhibition, a modest increase in K23/A1369 variant K$_{ATP}$ 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$_{ATP}$ channel to activation by intracellular acyl CoAs (11,13). K$_{ATP}$ 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$_{ATP}$ 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$_{ATP}$ channels expressing the K23/A1369 variant proteins is of importance. Free ATP inhibits K$_{ATP}$ 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$_{ATP}$ 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
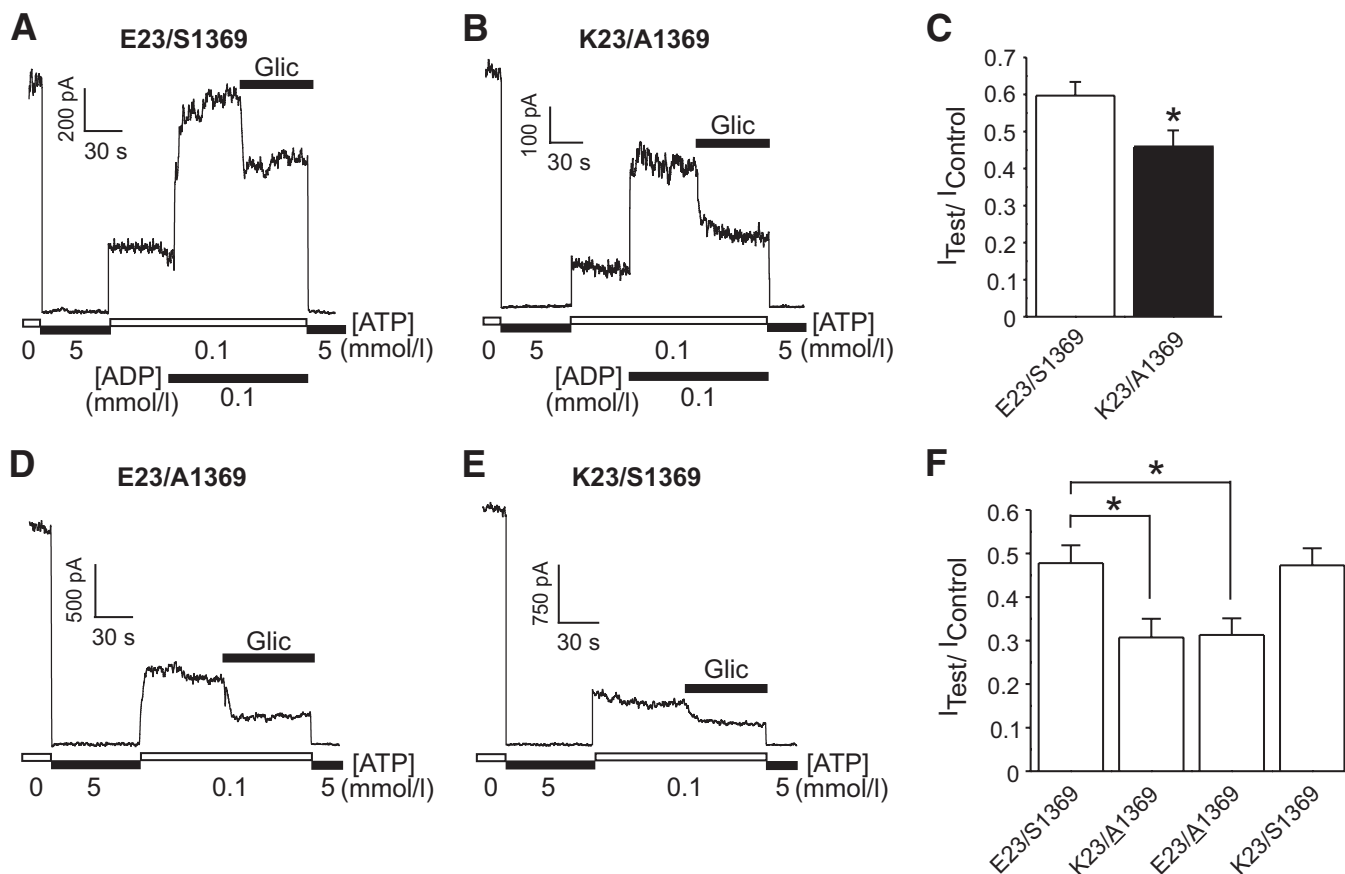
FIG. 4. The increased gliclazide sensitivity of K23/A1369 variant K_{ATP} channels is maintained in the presence of MgADP and is conferred upon the K_{ATP} channel complex by the *ABCC8* A1369 risk allele. *A* and *B*: Representative macroscopic current recordings showing the inhibitory effect of gliclazide (300 nmol/l) on the two variants in the presence of MgADP. *C*: Grouped data demonstrating that the K23/A1369 variant K_{ATP} channels are significantly more sensitive to gliclazide in the presence of MgADP than the E23/S1369 variant K_{ATP} channels. *n* = 10–12 patches per group. *D–F*: Representative current recordings and grouped data showing the increased gliclazide inhibitory effect is dependent on the presence of the *ABCC8* A1369 variant and not the *KCNJ11* K23 variant. *n* = 15 patches per group. *P < 0.05. Glic, gliclazide.

K23/A1369 variant K_{ATP} channel results from a direct effect of the *ABCC8* A1369 risk allele reducing ATP inhibition (9), perhaps via mild increases in the intrinsic K_{ATP} 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 K_{ATP} 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 K_{ATP} 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 K_{ATP} 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 K_{ATP} channel and identify the *ABCC8* A1369 risk allele as conferring this effect upon the K23/A1369 variant K_{ATP} 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 K_{ATP} 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 K_{ATP} 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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